



US Army Corps
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Base Realignment and Closure Program

The Results of a Laboratory Feasibility Study for the Biological Treatment of Umatilla Groundwater

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and Agnes Morrow

January 2012

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Final report

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Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000

Under Work Unit 33143

Abstract: The Umatilla Chemical Depot (UMCD) has been slated to close as an Army facility under the Base Realignment and Closure (BRAC) Program. One remaining environmental issue is a groundwater plume contaminated with explosives; the two most critical are 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX). Since 1994, a groundwater pump and treat system has operated at the site as a corrective measure for the contaminated groundwater. The effectiveness of this treatment system has plateaued, and it appears that the system will not meet the long-term treatment goals for the site. This study was conducted to evaluate the potential of bioremediation as a means of optimizing the performance of the groundwater treatment system. Groundwater from the site was collected through the groundwater pump and treat. Soil was collected from the wash out lagoon area, which is the primary source area for most of the contamination. These were used to set up microcosm studies to evaluate the biodegradation of the contaminants. Microcosms were set up using 1-liter Erlenmeyer flasks. The groundwater was spiked to about 1.2 and 0.8 mg/L of TNT and RDX, respectively. The flask had 200 g of Umatilla soil (some experiments had 50 g) and 500 mL of spiked groundwater. Various treatments were assessed; i.e., various organic amendments were used as co-substrates to stimulate the degradation of TNT and RDX. Nine amendments, as well as various unamended samples, were tested. The reactors were incubated over a 27-day period under an anaerobic hood. Removal of the contaminants was measured, as was the formation and removal of transformation products, changes in pH, Total Organic Carbon, Eh, and dissolved oxygen. TNT was relatively easy to degrade as it removed even many of the controls. Presumably the anaerobic conditions under the hood were enough to stimulate degradation. RDX, on the other hand, was more difficult to treat. The best amendments were molasses, corn syrup, emulsified oil (EOS), lactose, and whey.

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Contents

Figures and Tables.....	iv
Preface.....	vi
1 Introduction.....	1
Site Background.....	1
Literature Review – Biodegradation of Explosives in Groundwater.....	1
Objectives.....	3
Project Context.....	4
Umatilla Bioremediation Team.....	4
2 Materials and Methods.....	5
Approach.....	5
Aquifer Materials.....	5
Amendments.....	5
Reactors.....	6
Experiments.....	8
Sampling.....	9
Analyses.....	10
Reaction Rate Calculation.....	13
Data Quality Objectives.....	13
3 Results.....	15
Soil Data.....	15
Removal of TNT.....	17
Removal of RDX.....	20
Mass Balance of Explosives.....	23
Other Measurements.....	23
<i>Total Organic Carbon (TOC).....</i>	<i>23</i>
<i>Dissolved Oxygen Concentration (DO) and Eh.....</i>	<i>27</i>
<i>pH.....</i>	<i>29</i>
<i>Metals and Conductivity Data.....</i>	<i>30</i>
Cost and Sources of Amendments.....	34
4 Recommendations.....	39
References.....	40
Appendix A: Raw Explosive Analysis Data.....	42
Appendix B: Kinetic Analysis of Bioremediation Data.....	58
Report Documentation Page	

Figures and Tables

Figures

Figure 1. Location in former lagoon area where soil were collected.....	6
Figure 2. Mock up of bioreactor used in this study, an Erlenmeyer flask filled with soil and groundwater, spiked with contaminants and amendments.	7
Figure 3. Glove box set-up of experimental Run 1.	7
Figure 4. Close-up of reactors in experimental Run 1.	8
Figure 5. Temperature controlled sonicator used for extraction of explosives from aquifer sand/soil & a high pressure liquid chromatograph for explosives analysis.	11
Figure 6. TOC instrument used during project. It is useful for quantifying organic material in sand/soil and for analyzing additives in groundwater.	12
Figure 7. Ion chromatograph available for nutrient analysis.	12
Figure 8. Microwave digester and ICPOES for analysis of metals.....	13
Figure 9. Sieve plots of soils collected from UMCD for use in these studies.....	16
Figure 10. Run 1 TNT and breakdown products by amendment.	17
Figure 11. Run 2 TNT and breakdown products by amendment.	18
Figure 12. Run 1 RDX and breakdown products by amendment.....	21
Figure 13. Run 2 RDX and breakdown products by amendment.....	22
Figure B1. Plot of first-order function linearization TNT Run 1.....	60
Figure B2. Plot of first-order function linearization RDX Run 1.....	60
Figure B3. Plot of first-order function linearization TNT Run 2.	62
Figure B4. Plot of first-order function linearization RDX Run 2.....	62

Tables

Table 1. Amendment Sources.....	6
Table 2. Quantities of amendments used in experiments.	9
Table 3. Sample analytes, analysis method, and required volumes.	10
Table 4. Sampling schedule and required volumes for analysis (mL).....	10
Table 5. Data quality objectives for the project.	14
Table 6. Soil analysis for explosives.....	15
Table 7. Carbon sources sorted on TNT reaction rate.....	19
Table 8. Carbon sources sorted on RDX reaction rate.....	23
Table 9. Mass balance summary for TNT.....	24
Table 10. Mass balance summary for RDX.	25
Table 11. Run 1 TOC of all replicate reactors (mg/L).....	25
Table 12. Run 2 TOC of all replicate reactors (mg/L).....	26
Table 13. Run 1 Dissolved Oxygen (mg/L).....	27

Table 14. Run 2 Dissolved Oxygen (mg/L).....	28
Table 15. Run 1 Eh.....	30
Table 16. Run 2 Eh.....	31
Table 17. Run 1 pH.....	32
Table 18. Run 2 pH.....	33
Table 19. Run 1 Metals Analysis. Detection limit was 0.5 µg/L. BD means below detection.....	34
Table 20. Run 1 Conductivity µS/cm.....	35
Table 21. Run 2 Metals Analysis. Detection limit was 0.5 µg/L. BD means below detection.....	36
Table 22. Run 2 Conductivity µS/cm.....	37
Table 23. Amendment costs.....	38
Table 24. Sources for Key Amendments.....	38
Table A1. Run 1 TNT Raw Data.....	42
Table A2. Run 1 4 Amino 2,6-dinitrotoluene Raw Data.....	43
Table A3. Run 1 2 Amino 4,6 dinitrotoluene Raw Data.....	44
Table A4. Run 1 HMX Raw Data.....	45
Table A5. Run 1 RDX Raw Data.....	46
Table A6. Run 1 MNX Raw Data.....	47
Table A7. Run 1 DNX Raw Data.....	48
Table A8. Run 1 TNX Raw Data.....	49
Table A9. Run 2 TNT Raw Data.....	50
Table A10. Run 2 4 Amino 2,6-dinitrotoluene Raw Data.....	51
Table A11. Run 2 2 Amino 4,6 dinitrotoluene Raw Data.....	52
Table A12. Run 2 HMX Raw Data.....	53
Table A13. Run 2 RDX Raw Data.....	54
Table A14. Run 2 MNX Raw Data.....	55
Table A15. Run 2 DNX Raw Data.....	56
Table A16. Run 2 TNX Raw Data.....	57
Table B1. TNT Run 1.....	58
Table B2. RDX Run 1.....	59
Table B3. TNT Run 2.....	61
Table B4. RDX Run 2.....	61

Preface

This report describes experimental work conducted under funding provided by the Army Corps of Engineers, Seattle District, via a Military Interdepartment Purchase Request (MIPR). The Seattle District received funding for the project through the Base Realignment and Closure (BRAC) Program. Richard Wilson of the Seattle District monitored the progress of the project. The purpose of the project was to investigate biological treatment as a means for optimizing the treatment of explosives-contaminated groundwater at the Umatilla Chemical Depot (UMCD). This research effort was directed by Dr. David Gent under the oversight Dr. Victor F. Medina, P.E., Team Leader of the Environmental Security Engineering Branch. Dr. Heather Knotek-Smith, directed the laboratory experiments. Agnes Morrow provided laboratory support.

This report was prepared by Dr. Victor Medina, Dr. Heather Knotek-Smith, Dr. David Gent, P.E., and Agnes Morrow, all of Environmental Engineering Branch, Environmental Processes and Engineering Division (EPED), Environmental Laboratory (EL). Dr. Fiona Crocker and Dr. Mansour Zakakhani provided in-house review.

This study was conducted under the direct supervision of W. Andy Martin, Chief, Environmental Engineering Branch, and under the general supervision of Warren P. Lorentz, Chief, EPED, and Dr. Elizabeth C. Fleming, Director, EL.

At the time of publication of this report, COL Kevin J. Wilson was Commander of ERDC, and Dr. Jeffery Holland was Director.

1 Introduction

Site Background

The Umatilla Chemical Depot (UMCD) is located in Hermiston, Oregon, and served as a depot facility for explosive and chemical munitions from 1941 to 1990. In 1990, the UMCD was placed on the Base Realignment and Closure (BRAC) list as a facility to be closed. To prepare the facility for transition to unrestricted civilian use, the site must be restored from an environmental standpoint. Over the past 20 years, the non-chemical munitions at UMCD have been transferred to another facility or decommissioned. An incineration plant has been set up for the destruction of chemical munitions and much of the inventory has been treated. In addition, environmental issues at the facility have been addressed.

One remaining environmental issue is a groundwater plume contaminated with the explosives: 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX). In 1994, a groundwater pump and treat facility was installed at the site. Groundwater is extracted through three pumping wells (four were installed, but only three operate) and the explosives are removed using granular-activated carbon (U.S. Army Corps of Engineers, 2010). Over the past five years, treatment has stalled. This project was an investigation to explore the use of bioremediation as a means of optimizing.

The results of this project were used to design field studies that were planned to investigate the effectiveness of biotreatment for Umatilla groundwater, including a push-pull study and a study investigating the efficacy of using surface infiltration as a means to deliver amendments, called the Lagoon Amendment Pilot Project (LAPP).

Literature Review – Biodegradation of Explosives in Groundwater

There have been numerous studies based on bioremediation of high explosives in water. These studies often involve the addition of a co-substrate – a readily biodegradable material that stimulates microbial growth and consumes oxygen and other electron acceptors, causing conditions to become more reducing. Nitrated compounds can often be

degraded in anaerobic environments created by adding an organic co-substrate (Crawford, 2002).

Hoferkamp and Weber (2006) studied the kinetics of nitroaromatic reduction as a function of terminal electron acceptor in natural sediments. They found that lactate could serve as a source of organic carbon in sulfate, reducing sediment slurries. They also found that the reaction rates were significantly lower when the same slurries were amended with acetate. They concluded that the electron source and the system parameters, such as pH, play a determinant role in the reaction kinetics.

Krumholz et al. (1997) studied the transformations of TNT in groundwater aquifer slurries under methanogenic, sulfate-reducing and nitrate-reducing conditions. The groundwater used in these studies was collected from a methanogenic aquifer and was supplemented with the desired nitroaromatic compound to a final concentration of 100 μ M or 22.8 ppm in the case of TNT. The study found that the TNT was depleted three to five times faster under methanogenic conditions than it was under the sulfate and nitrate-reducing conditions. Anaerobic biodegradation of explosives by a methanogenic mixed culture was further investigated by Adrian et al. (2003). This work amended a basal medium containing 11.4 ppm TNT and 5.5 ppm RDX with ethanol, propylene glycol, butyrate or hydrogen gas as the electron donor. They found that all of the TNT and RDX were completely transformed in all of the bottles and concluded that “the addition of H₂ or electron donors that produced H₂ may be a useful strategy for enhancing the anaerobic biodegradation of explosives in contaminated ground water and soils.” Adrian and Arnett (2007) tested this conclusion on groundwater and contaminated soil (2.4 ppm RDX and 3.8 ppm TNT) using anoxic microcosms constructed from the contaminated environment amended with ethanol and propylene glycol. They found that the amendments enhanced the biotransformation of RDX in the explosive-contaminated soil but that the TNT was most likely chemically transformed.

Schaefer et al. (2007) studied the biodegradation of RDX (5 mg L⁻¹) and HMX (1 mg L⁻¹) when co-mingled with nitrate (4 mg L⁻¹) and perchlorate (5 mg L⁻¹). They utilized an emulsified vegetable oil as a substrate and found that it effectively promoted the biological reduction of nitrate, RDX, and perchlorate in the microcosm experiments and all four target contaminants in the column flow-through studies.

A soil slurry reactor was used by Boopathy et al. (1998) to study the bioremediation of a TNT-contaminated soil using molasses as a cosubstrate. The TNT concentration in the soil ranged from 4,000 to 12,000 mg kg⁻¹ and the molasses solution was 0.3%. The tests resulted in the transformation of TNT by batch treatment and complete degradation of TNT in the semicontinuous treatments. Boopathy and Manning (1999) continued this work by adding surfactant with the molasses; in this way, they were able to completely transform the TNT. The addition of the surfactant most likely keeps the TNT mineralization products from binding to the slurry and therefore remains bioavailable and can be completely broken down. Additionally, Boopathy and Manning (2000) demonstrated the ability of a 0.3% molasses solution to serve as a co-substrate for the degradation of RDX. In this experiment, a soil slurry reactor was used to decrease an initial soil concentration of 7000 mg kg⁻¹ RDX by 98% in four months.

Waisner et al. (2002) used a tagged RDX compound to maximize the mineralization of the compound within soil-water slurries under anaerobic and aerobic conditions. It was found that the RDX (202 µg L⁻¹) was completely mineralized when acetate (92 mg L⁻¹) was added to the test reactors. Wani and Davis (2003) also saw the complete removal of RDX (1.8 µg L⁻¹) using acetate (500 mg L⁻¹) as a carbon source. This work studied the degradation in flow-through columns packed with aquifer material.

Objectives

Based on the studies presented above, it appeared that biodegradation of explosives stimulated by the addition of an organic co-substrate could be an effective approach for treating low levels of explosives concentrations at the Umatilla Army Depot. The objectives of this study were:

- to evaluate various amendments and amendment combinations for their ability to promote biodegradation;
- to assess whether biodegradation can meet treatment goals (2 µg L⁻¹ for RDX and TNT);
- to provide recommendations for the best amendments for subsequent field experiments, taking into account cost considerations; and
- to provide kinetic data for affiliated modeling efforts.

Project Context

This project will provide data for two other related field projects. When combined, these data will provide information to evaluate the efficacy of bioremediation to meet treatment goals at Umatilla. Further, these combined projects will provide the basis for the design of full-scale applications. The two additional projects include a push-pull test, which is conducted *in situ*, allowing for dispersion effects to be evaluated. The other project will deliver amendments into the aquifer via an infiltration gallery.

Umatilla Bioremediation Team

A team was assembled to assess the feasibility of applying bioremediation as a means of optimizing the Umatilla Groundwater treatment system. The Seattle District coordinated the overall effort, was the primary liaison with the various regulatory agencies, and was in charge of the push-pull testing. The U.S. Army Engineer Research and Development Center (ERDC) Environmental Laboratory (EL) provided laboratory experimentation and coordinated any analytical work associated with field endeavors. The Army Environmental Command designed an infiltration study which will be implemented by the Seattle District. The ERDC Coastal and Hydrology Laboratory (CHL) provided modeling support for the team. The Environmental Protection Agency (EPA) and the Oregon Department of Environmental Quality (ODEQ) provided oversight throughout the project.

2 Materials and Methods

Approach

This study used batch reactors to study the degradation of explosives stimulated by adding organic co-substrates. Reactors that allowed for multiple sampling were designed, constructed and used. Reactor preparation, incubation, and sampling were conducted under an anaerobic hood. The goal of this project was to evaluate in a controlled laboratory setting potential amendments that could be added to explosives-contaminated groundwater at the Umatilla Army Depot to simulate biodegradation of these contaminants.

Aquifer Materials

Groundwater for the experiments was collected on-site from the influent spigot of the groundwater treatment system by the Seattle District. The reactors were spiked to have an RDX concentration of 800 and a TNT concentration of 1200 $\mu\text{g L}^{-1}$. Spiking allowed the experiments to be conducted more quickly since the analytical approach will not require any concentration steps. Spiking also allowed us to study the formation and degradation of breakdown products.

Time and cost constraints did not allow for the collection of actual aquifer material from the site. Instead, ERDC personnel collected soil material from the drainage lagoon site as a surrogate for actual groundwater material. Three buckets of soil from this area were collected (Figure 1). Approximately one foot of soil was removed from the three locations prior to the sample collection to minimize the amount of vegetative material. The soil had the appearance of basalt rocks of various sizes with sand.

Amendments

Nine amendments were tested in this study. They were chosen based on previous results for the treatment of explosives described in the literature. The general sources of amendments used in this study are listed in Table 1.



Figure 1. Location in former lagoon area where soil were collected.

Table 1. Amendment Sources.

molasses	Grandma's Molasses
high fructose corn syrup	Kroger brand
lactose	Columbia River Processors, Boardman, OR
cheese whey	Columbia River Processors, Boardman, OR
lactate	JWR Bioremediation
ethanol	Everclear (95%)
emulsified soybean oil	EOS Remediation
benzoic acid	technical grade
sodium acetate	technical grade

Reactors

A mock-up using a 1-liter Erlenmeyer flask was used to test an appropriate mixture of soil and water (Figure 2). Based on this mock-up, the bench scale treatability study was conducted in prepared reactors using 1 liter amber screwtop bottles with 200 g of aquifer material (some reactors in test 2 used 50 g and are labeled as 50 g reactors) and 500 mL of amended/spiked groundwater (Figures 3 and 4). All reactors were sealed with a screw top and incubated in an anaerobic glove box on a shaker at room temperature.



Figure 2. Mock up of bioreactor used in this study, an Erlenmeyer flask filled with soil and groundwater, spiked with contaminants and amendments.



Figure 3. Glove box set-up of experimental Run 1.



Figure 4. Close-up of reactors in experimental Run 1.

Experiments

Due to size limitations in the glove box, the work was carried out in two experimental runs. The co-substrates (which were chosen based on studies documented in the literature or after personal communication with experts) tested in Run 1 were:

- high-fructose corn sugar (based on promising results obtained using soft drink by-products described by Ira May)
- molasses (Boopathy et al. 1998)
- lactose (the remaining filtrate of cheese whey – see below)
- emulsified oil (EOS) (Schaefer et al. 2007)
- acetate (Waisner et al. 2002 and Wani and Davis, 2003) and
- a control consisting of unamended well water and sediment, but not autoclaved.

Experimental Run 2 tested the following treatments:

- Sodium Lactate (Hoferkamp and Weber 2006)
- Liquid cheese whey (promising results were reported by Shaw Environmental, Hatzinger, Pers. Comm.)

- Benzoic acid chosen due to promising results for treatment of perchlorate (Stroo and Norris 2008)
- Ethanol (Krumholz et al. 1997)
- A control consisting of unamended well water and sediment, but not autoclaved.
- An autoclaved control (autoclaved twice one day apart and a third time one week later and two times two weeks later one day apart)
- A low solids (50 g/500 mL) control, not autoclaved
- A low solids (50 g/500 mL) treatment with Lactose

Studies by Wani and Davis (2003) indicated that 500 mg L⁻¹ of acetate was effective for complete RDX degradation. We expected levels of other amendments to be near the same level. Table 2 summarizes the quantities of each amendment used in the experiment.

Table 2. Quantities of amendments used in experiments.

Amendment	Quantity Added	mol/L	Initial TOC	Initial St Dev
sodium acetate	2 g/L	0.0244	438	12
lactate	6 mL/L	0.0712	630	14
molasses	3 g/L	0.0149	977	256
corn syrup	4 g/L	0.0167	1002	300
ethanol	10 mL/L	0.1629	1638	173
benzoic acid	0.1 g/L	0.0008	47	13
EOS	10 mL/L	0.0346	539	87
lactose	20 mL/L	?	1646	181
whey	6 mL/L	?	211	11

Sampling

For experiment 1, samples were taken at the following intervals: 0 days, 1 day, 2, 5, 8, 12, 15, 20, and 27 days. This timeframe was sufficient to see degradation as well as to allow evaluation of any lag phase. A lag phase had been found in studies on the sequential biodegradation of TNT and RDX mixtures (Sagi-Ben Moshe et al. 2008) in groundwater.

Table 3 summarizes measurements that were conducted on the liquid samples throughout the experiment. Table 4 summarizes the analytical frequency and sample volumes needed. At the end of the experiment, the aquifer soil was also extracted for explosives.

Table 3. Sample analytes, analysis method, and required volumes.

Analysis	Method	Volume Needed
RDX, TNT and transformation products using High Pressure Liquid Chromatography (Figure 5)		
Other explosives found on the analyte list of EPA Method 8330b	EPA 8330 (USEPA 2006)	2(2mL)=4
pH	EPA 150.2 (USEPA 1982b)	0
Oxygen Reduction Potential (ORP)	ASTM D1498-00	0
Total Organic Carbon (TOC) (Figure 6)	EPA 9060A (USEPA 2004)	5-10 mL
Nitrate (Figure 7)	EPA 300.0 (USEPA 1993)	2 mL
Dissolved Iron and Manganese using ICPOES (Figure 8)	EPA 200.7 (USEPA 1994)	5 mL
Conductivity	EPA 120.1 (USEPA 1982a)	0

Table 4. Sampling schedule and required volumes for analysis (mL).

Day	RDX, TNT, Transformation Products	TOC	Nitrate	Fe Mg	Total Volume
0	4	5	2	5	16
1	4				4
2	4				4
5	4				4
8	4				4
12	4	10	2	5	21
15	4				4
20	4				4
27	4	10	2	5	21

Based on the results of experiment 1, it was determined that the sampling frequency for experiment 2 could be abbreviated somewhat. Sampling was conducted at 0, 2, 7, 15, and 24 days.

Analyses

The sand/soil samples were homogenized, then extracted and analyzed for the following:

- RDX, TNT, and transformation products using extraction and analysis specified in EPA method 8330.
- Other explosives constituents listed in the analyte list of EPA method 8330b.
- pH using EPA Method 150.2.
- Total organic carbon (TOC) using EPA Method 415.1.
- Iron, manganese, and calcium using EPA Method 3052 (Microwave Assisted Acid Extraction) for sample digestion and EPA Method 415.1 (Inductively Coupled Plasma with Optical Emission Spectrophotometry (ICPOES) for analysis.
- Grain size and soil characteristics.

In addition, groundwater collected from the site was mixed into a common container, then analyzed for the following:

- RDX, TNT, and transformation products using EPA method 8330, modified to include nitroso products from RDX (High Pressure Liquid Chromatography) (Figure 5)



Figure 5. Temperature controlled sonicator used for extraction of explosives from aquifer sand/soil & a high pressure liquid chromatograph for explosives analysis.

- Other explosives constituents listed in the analyte list of EPA method 8330b.
- pH using EPA Method 150.2
- Oxidation Reduction Potential (ORP) using ASTM D1498-00
- Total organic carbon (TOC) (EPA Method 415.1) or measurement of amendment (Figure 6)
- Nitrate using EPA Method 300.0 (Ion Chromatography) (Figure 7)
- Dissolved iron and manganese using EPA Method 200.7 (ICPOES) (Figure 8)



Figure 6. TOC instrument used during project. It is useful for quantifying organic material in sand/soil and for analyzing additives in groundwater.



Figure 7. Ion chromatograph available for nutrient analysis.



Figure 8. Microwave digester and ICPOES for analysis of metals.

- Conductivity using EPA Method 120.1
- Suspended solids using EPA method 160.2

The same analyses were conducted on samples collected during the experiment and on the soil afterwards.

Reaction Rate Calculation

The degradation rate was assumed to be first order (Eweis et al. 1998). Determination of the reaction rate was based on Equation 1. A linearized form of this equation is shown in Equation 2. A plot of this function results in k , the slope of the curve. The half life is defined as the time needed to biodegrade 50% of the contaminant initially present. The equation used to calculate this value is shown in Equation 3.

$$C = C_0 e^{-kt} \quad (1)$$

$$\ln \frac{C_0}{C} = kt \quad (2)$$

$$t = -\frac{1}{k} \ln \frac{C}{C_0} = -\frac{\ln(0.5)}{k} \quad (3)$$

Data Quality Objectives

Table 5 gives the data quality objectives set by the bioremediation team for the project. The experimental plan met all of the objectives, providing the basis for amendment selection and allowing for the quantification of degradation rates. The experiments also evaluated transformation products.

Table 5. Data quality objectives for the project.

Objective	Data Gap	Method	Data Use/Decision
ERDC Vicksburg Laboratory Treatability Testing <ul style="list-style-type: none">• Screen multiple amendments• Determine RDX and TNT transformation rates• Identify potential transformation products	Cost-effective amendment(s) Degradation rates Final extent of degradation Transformation products	Batch tests Analytes: RDX, TNT, and transformation intermediates, dissolved Fe, O ₂ , SO ₄ , ORP, NO ₃ , TOC (or substrate), pH	Identify amendments for push-pull test Quantify rate, evaluate cost

3 Results

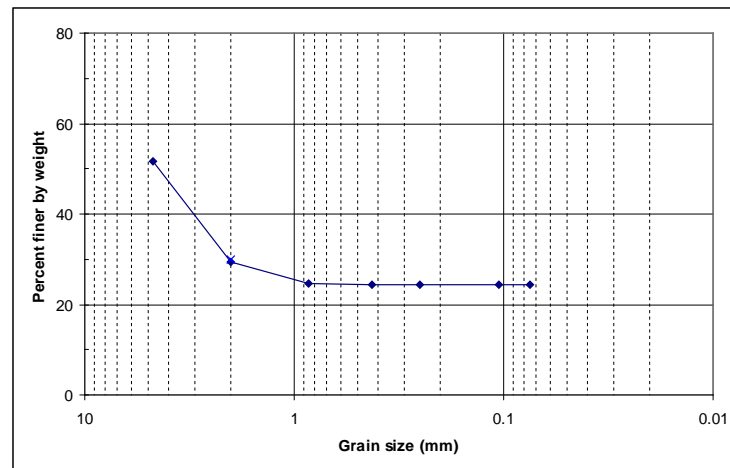
Soil Data

Table 6 summarizes explosives data collected from homogenized samples from the lagoon area at the UMCD. Various explosives and transformation products were detected, including HMX, RDX (although no transformation products were found), TNT, 1,3,5-Trinitrobenzene (TNB) and 4-amino-2,6-dinitrotoluene (4ADNT). Soils from the three buckets were mixed and homogenized for the actual study. Equal masses from each bucket were placed on a stainless steel tray. The soils were well mixed then sieved through a #10 (2 mm) sieve to remove the largest rocks before placement in the reactors. Figure 9 summarizes the grain sieve analysis of the buckets of soil.

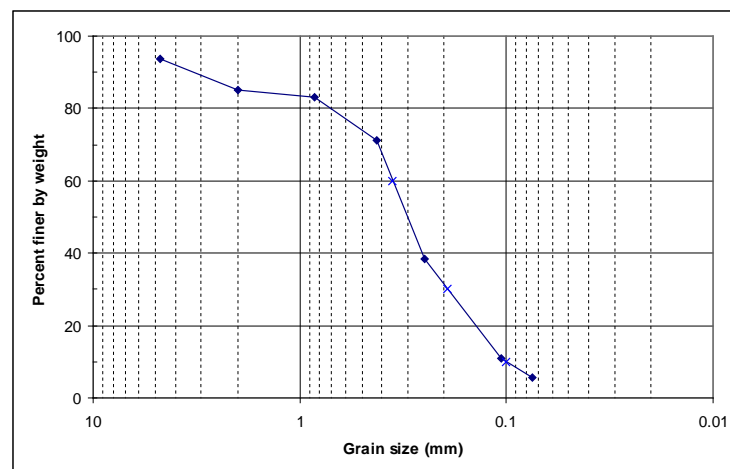
Table 6. Soil analysis for explosives.

Analyte	Bucket 1 µg/kg	Bucket 2 µg/kg	Bucket 3 µg/kg
HMX	6610	9690	3920
TNX	<19.8	<19.8	<19.9
DNX	<19.8	<19.8	<19.9
MNX	<19.8	<19.8	<19.9
RDX	<19.8	294	429
1,3,5-Trinitrobenzene	121	9400	14300
1,3-Dinitrobenzene	<19.8	<19.8	<19.9
Nitrobenzene	<19.8	<19.8	<19.9
2,4,6-Trinitrotoluene	908	885	1390
4-Amino-2,6-dinitrotoluene	<19.8	<19.8	<19.9
2-Amino-4,6-dinitrotoluene	<19.8	147	61.9
2,6-Dinitrotoluene	<19.8	<19.8	<19.9
2,4-Dinitrotoluene	<19.8	<19.8	<19.9
2-Nitrotoluene	<19.8	<19.8	<19.9
4-Nitrotoluene	<19.8	<19.8	<19.9
3-Nitrotoluene	<19.8	<19.8	<19.9

Bucket 1 Sieve Analysis



Bucket 2 Sieve Analysis



Bucket 3 Sieve Analysis

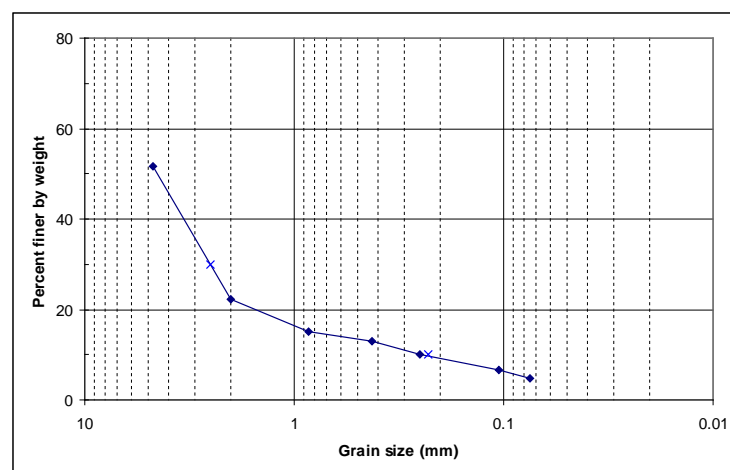


Figure 9. Sieve plots of soils collected from UMCD for use in these studies.

Removal of TNT

Figures 10 and 11 summarize TNT removal from experimental Runs 1 and 2, respectively. Appendix A contains full data tables with the values of the triplicate analyses. Table 7 contains first order degradation rate information on the various treatments (calculations and graphs deriving this data are in Appendix B). Substantial TNT removal was found in every reactor tested, including the controls. One hypothesis developed by the team is that TNT removal in the controls results from the reducing condition generated simply from being incubated in the anaerobic hood. This suggests that TNT removal should be easily achieved with even a modest decrease in the oxidation potential in the aquifer. Removal occurred even in the autoclaved

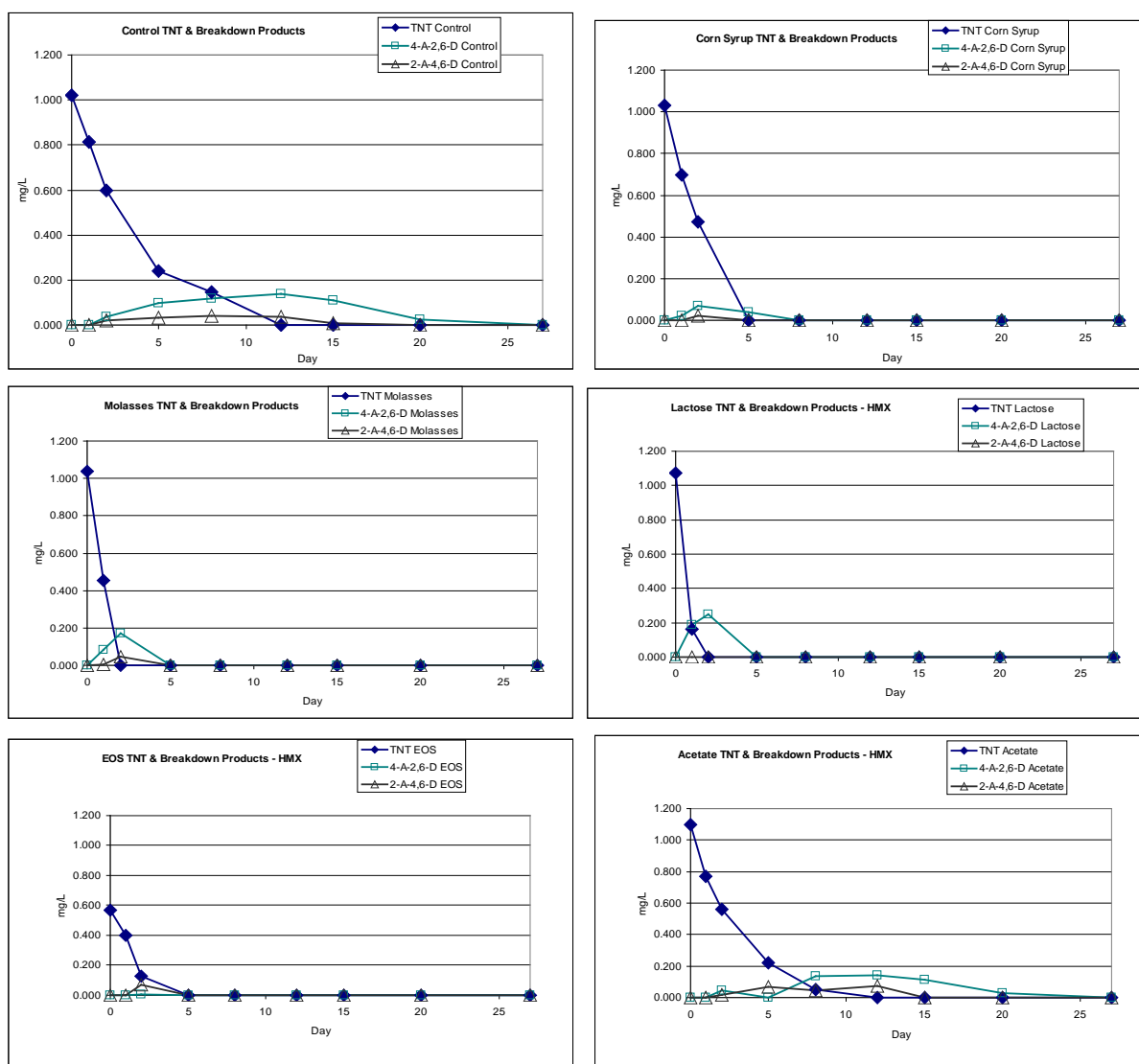


Figure 10. Run 1 TNT and breakdown products by amendment.

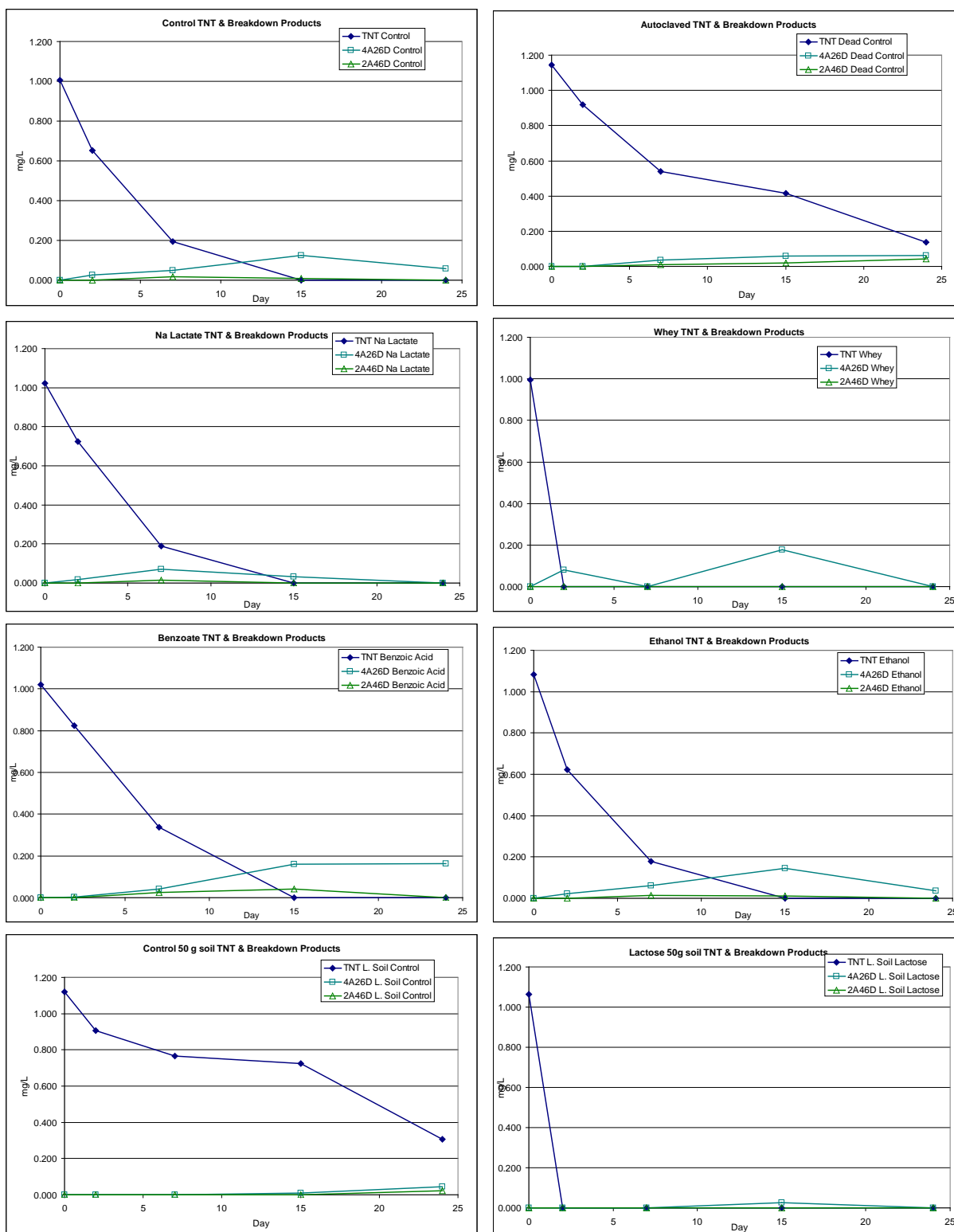


Figure 11. Run 2 TNT and breakdown products by amendment.

Table 7. Carbon sources sorted on TNT reaction rate.

Treatment	TNT	
	Reaction Rate (day ⁻¹)	Half Life (days)
L. Soil Lactose	3.138	0.221
Whey	3.105	0.223
Lactose	1.879	0.369
Molasses	0.827	0.838
EOS	0.674	1.028
Corn Syrup	0.391	1.772
Acetate	0.368	1.883
Ethanol	0.258	2.685
Control Run 1	0.254	2.725
Na Lactate	0.236	2.942
Control Run 2	0.234	2.967
Benzoate	0.154	4.489
Autoclaved	0.084	8.232
L. Soil Control	0.048	14.531

control (Figure 10). This reactor had a slower removal rate compared to the unautoclaved control overall; however, by the end of the 27-day experiment, about 90% of the TNT had been removed. Autoclaving is commonly used to kill microorganisms, but it is common to find that soil microbes are resistant to complete sterilization.

The organic substrates generally increased the removal rate of the TNT. In Run 1, the most rapid removal of the TNT occurred with molasses and lactose substrates. Corn syrup and EOS also had relatively rapid removal of TNT. Acetate appeared to have about the same TNT removal as the control. In Run 2, TNT degradation rates with the whey amendment were similar to that of molasses and lactate. The removal of TNT by the other substrates used in Run 2 was similar to that of the control.

Detection limits of the Run 1 study were 10 µg L⁻¹ for all of the data except for the last (27-day) data point. The detection limit for that point was 2 µg L⁻¹. All of the Run 1 amended treatments successfully reached levels below the 2 µg L⁻¹ level at this time. Similarly, the detection limits for the Run 2 study were 10 µg/L except for the last (24-day) sample, for which a detection limit of 4 µg L⁻¹ was obtained.

The graphs do not have error bars. However, standard deviations are given in the data tables in Appendix A. These indicate that the standard deviations are small compared the average measurements values. For example, for the first two TNT data points for lactose, the relative standard deviation (RSD) (std dev/average) were 0.8 and 6.7% respectively.

In each case, TNT removal is accompanied by the formation of amino transformation products (4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene). Fortunately, these compounds were subsequently degraded in most cases. Lactose and molasses appeared to be particularly effective at removal of transformation products.

Removal of RDX

Figures 12 and 13 are graphs of RDX removal and breakdown product formation (complete data tables are in Appendix A) and Table 8 summarizes calculated degradation rates and half-lives for various RDX treatment approaches (calculations and graphs deriving this data is in Appendix B). Removal in all the controls tested was minimal (<20% during the 27-day experiment in all cases). Two amendments showed no appreciable RDX degradation (<20% over the 27-day experiment): acetate and benzoic acid. Lactate and ethanol had similar patterns, showing minimal degradation during the first four sampling periods (15 days), but having a substantial drop during the last sampling period (24 days). The best performing amendments in terms of RDX removal were lactose (testing in both a 200 g solids/500 mL solution and a 50 g/500 mL solution set ups), EOS, molasses, whey, and corn syrup. Each of these reached RDX below 10 $\mu\text{g L}^{-1}$ detection limit within 15 or less days.

Like the TNT results, review of the data (Appendix A) indicates that variation of triplicate samples was generally small. For the first three data points for lactose RDX degradation, RSDs ranged from 0.7 to 3.4%. The fourth data point has an RSD of 124%, but this was the last point where any detection of RDX was found, the average was 8 $\mu\text{g L}^{-1}$ with a std. dev. of 10 $\mu\text{g L}^{-1}$. The detection limits for RDX were the same as those for TNT.

In most cases, comparing the TNT and RDX plots for given contaminants suggest that RDX degradation begins or accelerates after TNT is largely removed from the system. EOS provides a sharp result. TNT degradation in EOS began immediately, but RDX degradation was delayed for the first three sampling periods. After the TNT was largely removed, RDX

degradation occurred rapidly. This is consistent with results found by Sagi-Ben Moshe et al. (2009). The pattern was found with other substrates, although it appeared perhaps not as strongly the EOS result.

We tracked three nitroso-substituted, transformation products of RDX: MNX, DNX, and TNX. As RDX was degraded, these compounds were sequentially formed, then degraded. Degradation did not reach non-detect levels in most cases; however, it was clear that degradation was occurring.

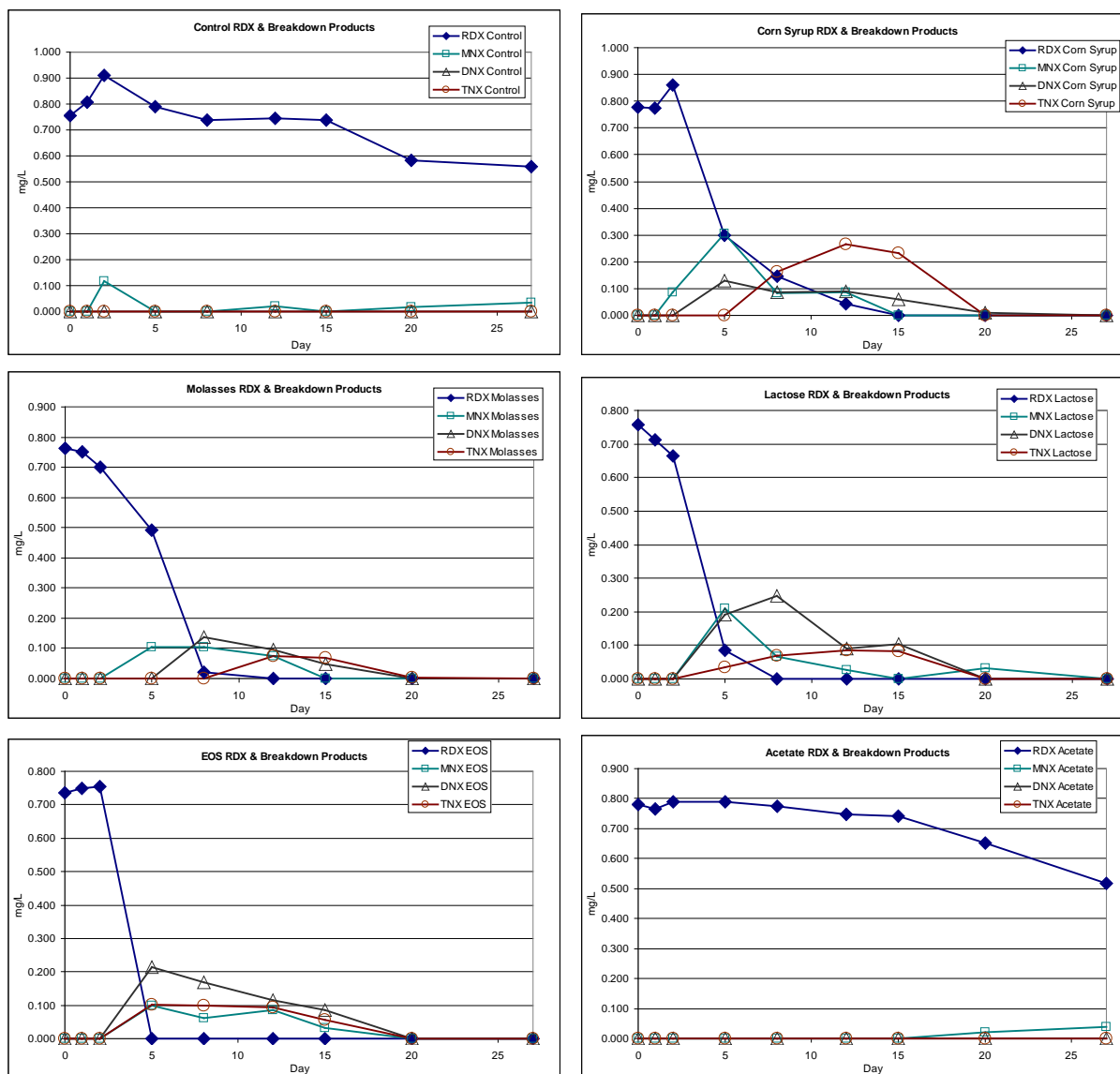


Figure 12. Run 1 RDX and breakdown products by amendment.

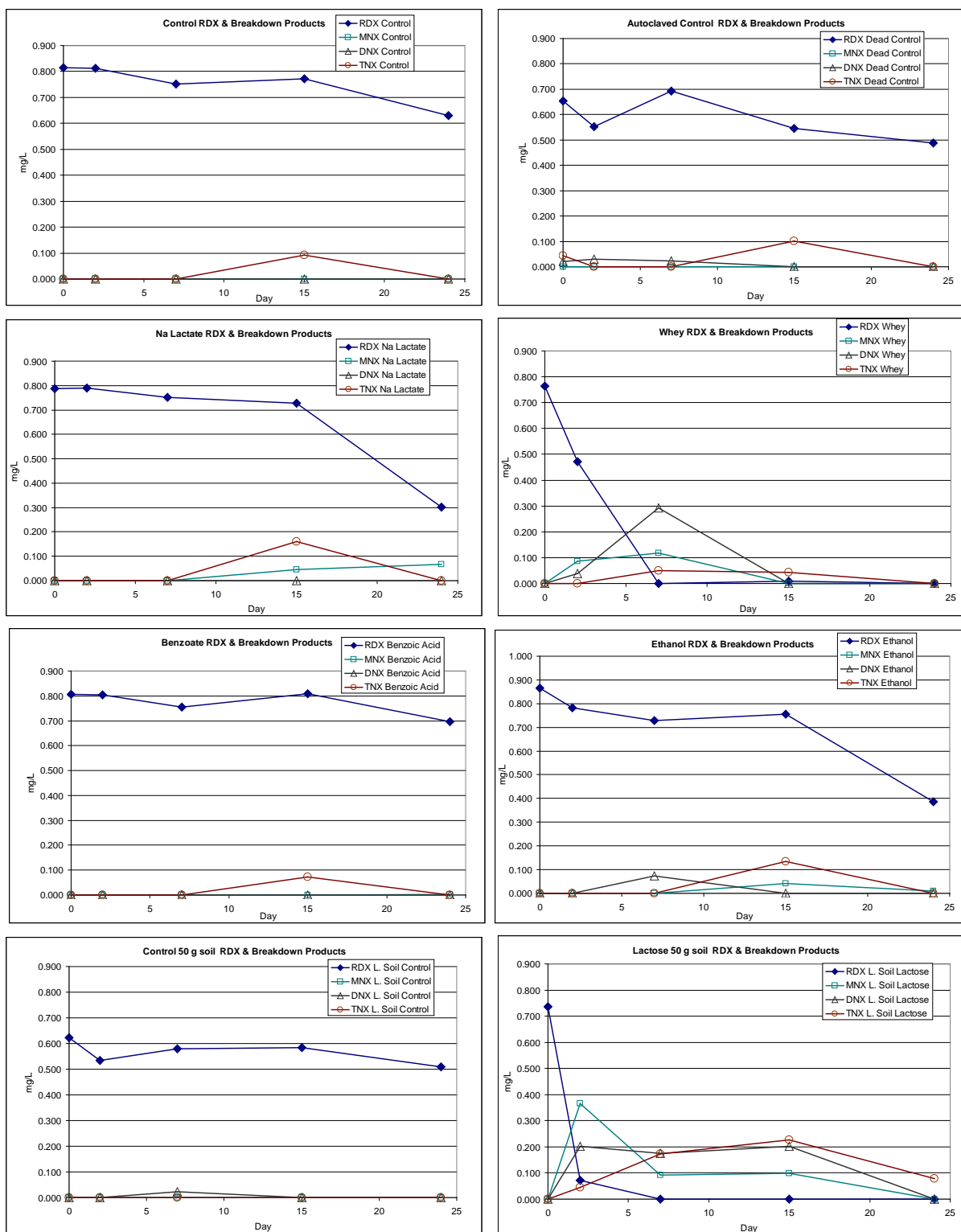


Figure 13. Run 2 RDX and breakdown products by amendment.

Table 8. Carbon sources sorted on RDX reaction rate.

	RDX	
	Reaction Rate (day ⁻¹)	Half Life (days)
L. Soil Lactose	1.158	0.598
EOS	0.983	0.705
Lactose	0.377	1.838
Molasses	0.326	2.124
Whey	0.240	2.889
Corn Syrup	0.220	3.146
Na Lactate	0.029	24.151
Ethanol	0.027	25.961
Autoclaved	0.011	61.340
Acetate	0.010	70.015
Control Run 2	0.009	78.767
Control Run 1	0.008	83.51
L. Soil Control	0.008	88.865
Benzoate	0.005	150.684

Mass Balance of Explosives

Table 9 summarizes mass balance information for TNT during both Test 1 and 2. If the Mass After Treatment was higher than the Time 0 Mass in the Soil, then some of the contaminant removal could be attributed to adsorption. In three cases, higher TNT concentrations were found in the soil after the treatment: the dead control, the soil control and the whey amendment treatment. All had higher TNT concentrations, and all were tested in Test 2. However, in each of these cases, the amount of accumulation was far less than the TNT removed from solution during the experiment.

Table 10 summarizes the mass balance for RDX. The mass of RDX after treatment was less than the time 0 concentration in each experiment.

Other Measurements

Total Organic Carbon (TOC)

There are several potential sources of TOC in the reactor systems, including native organic material (humic acids, plant material, etc) in the soil and groundwater used in the experiment and microorganisms themselves. However, the amendments added to the reactors were expected to be the greatest contributors to TOC. Therefore, TOC was used as a surrogate

measurement for amendment concentration. Tables 11 and 12 summarize the TOC of the reactors for Runs 1 and 2, respectively. As expected, adding the organic co-substrates increased the TOC in the reactor systems as compared to the control, from approximately 20 mg/L to 439 (acetate) up to 1646 mg/L (lactose). No consistent pattern was found in terms of TOC concentrations in the amended reactors over time. Corn syrup, molasses, and lactose (200 g solids/500 mL solution) all appeared to have an appreciable drop from their time 0 samples compared to subsequent measurements. This pattern suggests amendment loss over time due to its use as a food source, causing losses due to conversion to CO₂. EOS showed an appreciable drop from the time 0 to time 12 sample, but the 27-day sample had an increased value close to the concentration of the time 0 sample. For acetate, whey, and the 50g/500mL lactose reactor, the TOC concentrations remained more or less constant during the 27-day experiment. Several amendment reactors actually had an increase in TOC over time, including benzoic acid, ethanol, and sodium lactate.

Table 9. Mass balance summary for TNT.

TNT all units are mg						
	Time 0 Mass in Soil	Mass After Treatment	Mass Balance	Accumulation? (after > Time 0)	Mass of TNT in Solution	Is Accum. > TNT in Solution?
<i>Test 1</i>						
Control	0.083	0.071	-0.012	no	0.5	N/A
Corn Syrup	0.083	0.023	-0.060	no	0.5	N/A
Molasses	0.083	0.029	-0.054	no	0.5	N/A
Lactose	0.083	0.033	-0.050	no	0.5	N/A
EOS	0.083	0.032	-0.051	no	0.5	N/A
Acetate	0.083	0.072	-0.011	no	0.5	N/A
<i>Test 2</i>						
Control	0.083	0.078	-0.005	no	0.5	N/A
Benzoic Acid	0.083	0.076	-0.007	no	0.5	N/A
Ethanol	0.083	0.063	-0.020	no	0.5	N/A
Lactose	0.083	ND	-0.083	no	0.5	N/A
Dead Control	0.083	0.104	0.021	yes	0.5	no
Soil Control	0.083	0.131	0.048	yes	0.5	no
Whey	0.083	0.112	0.029	yes	0.5	no
Na Lactate	0.083	0.046	-0.037	no	0.5	N/A

*ND=non detect *N/A = not applicable

Table 10. Mass balance summary for RDX.

RDX all units are mg						
	Time 0 Mass in Soil	Mass After Treatment	Mass Balance	Accumulation? (after > Time 0)	Mass of RDX in Solution	Is Accum > RDX in Solution?
<i>Test 1</i>						
Control	0.901	0.157	-0.744	no	0.4	N/A
Corn syrup	0.901	0.027	-0.874	no	0.4	N/A
Molasses	0.901	0.037	-0.864	no	0.4	N/A
Lactose	0.901	0.049	-0.852	no	0.4	N/A
EOS	0.901	0.046	-0.855	no	0.4	N/A
Acetate	0.901	0.091	-0.810	no	0.4	N/A
<i>Test 2</i>						
Control	0.901	0.198	-0.703	no	0.4	N/A
Benzoic Acid	0.901	0.172	-0.729	no	0.4	N/A
Ethanol	0.901	0.126	-0.775	no	0.4	N/A
Lactose	0.901	0.076	-0.825	no	0.4	N/A
Dead Sea	0.901	0.652	-0.249	no	0.4	N/A
Soil Control	0.901	0.508	-0.393	no	0.4	N/A
Whey	0.901	0.098	-0.803	no	0.4	N/A
Na Lactate	0.901	0.065	-0.836	no	0.4	N/A

Table 11. Run 1 TOC of all replicate reactors (mg/L).

Day	Control			Average	St Dev
0	26	17	18	20	4
12	16	18	15	16	1
27	28	23	21	24	3
Day	Corn Syrup			Average	St Dev
0	1,408	689	911	1,002	300
12	573	535	536	548	18
27	547	550	704	601	73
Day	Molasses			Average	St Dev
0	754	840	1,335	977	256
12	556	636	559	584	37
27	551	625	544	573	37
Day	Lactose			Average	St Dev
0	1,494	1,544	1,901	1,646	181
12	1,146	1,032	1,283	1,154	103
27	1,176	1,036	1,275	1,162	98
Day	EOS			Average	St Dev
0	603	599	416	539	87
12	205	189	178	191	11

27	527	435	348	436	73
Day	Acetate			Average	St Dev
0	435	453	424	438	12
12	439	443	442	441	2
27	420	442	432	432	9

Table 12. Run 2 TOC of all replicate reactors (mg/L).

Day	Control			Average	St Dev
0	27.8	9.3	7.4	14.8	11.2
15	48.0	38.9	40.3	42.4	4.9
27	44.9	45.7	40.9	43.8	2.5
Day	Benzoic			Average	St Dev
0	36.1	42.1	61.8	46.7	13.5
15	87.2	112.4	110.3	103.3	14.0
27	92.7	120.2	117.2	110.0	15.1
Day	Ethanol			Average	St Dev
0	1785.6	1680.6	1447.7	1638.0	172.9
15	3040.8	3066.0	2940.0	3015.6	66.7
27	3063.9	3089.1	3034.5	3062.5	27.3
Day	Whey			Average	St Dev
0	204.1	205.7	224.1	211.3	11.1
15	349.9	329.3	10.2	229.8	190.4
27	289.6	338.3	302.0	310.0	25.3
Day	Na Lactate			Average	St Dev
0	641.2	614.6	633.5	629.8	13.7
15	1140.7	1061.1	1176.0	1126.0	58.8
27	1086.8	1089.5	1072.7	1083.0	9.0
Day	Lactose 50 g soil			Average	St Dev
0	719.1	683.5	737.6	713.4	27.5
15	846.7	872.1	872.8	863.9	14.9
27	851.1	886.4	887.5	875.0	20.7
Day	Autoclaved Control			Average	St Dev
0	26.1	25.9	20.6	24.2	3.1
15	45.2	42.6	47.1	45.0	2.3
27	46.5	44.9	26.4	39.3	11.2
Day	Control 50 g soil			Average	St Dev
0	20.3	63.9	6.9	30.4	29.8
15	35.9	34.4	43.8	38.0	5.1
27	36.8	37.2	34.8	36.3	1.3

Dissolved Oxygen Concentration (DO) and Eh

Tables 13 and 14 summarize the Dissolved Oxygen data for Runs 1 and 2 respectively. Initial DO values were, unfortunately, not triplicated for time 0 samples for Run 1; this was done due to the rapid variability in the reactor. A steady state measurement was not possible because probe readings were unstable. The control had a relatively low initial DO of 0.4 mg/L. The measurements were conducted under the anaerobic hood, which is the likely reason for the low DO levels. Initial DO measurements of other treatments ranged from 0.4 to 0.9 mg/L.

Table 13. Run 1 Dissolved Oxygen (mg/L).

Day	Control			Average	St Dev
0	0.36			0.36	
12	0.67	0.15	0.12	0.31	0.31
27	0.07	0.06	0.53	0.22	0.27
Day	Corn Syrup			Average	St Dev
0	0.40			0.40	
12	0.32	0.31	0.17	0.27	0.08
27	0.66	0.52	0.40	0.53	0.13
Day	Molasses			Average	St Dev
0	0.55			0.55	
12	0.17	0.17	0.22	0.19	0.03
27	0.10	0.16	0.14	0.13	0.03
Day	Lactose			Average	St Dev
0	0.50			0.50	
12	0.11	0.11	0.26	0.16	0.09
27	0.02	0.20	0.20	0.14	0.10
Day	EOS			Average	St Dev
0	0.88			0.88	
12	0.04	0.17	0.08	0.10	0.07
27	0.43	0.53	0.49	0.48	0.05
Day	Acetate			Average	St Dev
0	0.78			0.78	
12	0.24	0.27	0.20	0.24	0.04
27	0.71	0.74	0.60	0.68	0.07

Table 14. Run 2 Dissolved Oxygen (mg/L).

Day	Control			Average	St Dev
0					
15	0.26	0.31	0.21	0.26	0.05
27	0.35	0.22	0.20	0.26	0.08
Day	Benzoic acid			Average	St Dev
0					
15	0.27	0.37	0.18	0.27	0.10
27	0.23	0.27	0.21	0.24	0.03
Day	Ethanol			Average	St Dev
0					
15	0.00	0.01	0.12	0.04	0.07
27	0.29	0.18	0.02	0.16	0.14
Day	Whey			Average	St Dev
0					
15	0.06	0.15	0.00	0.07	0.08
27	0.12	0.27	0.18	0.19	0.08
Day	Na Lactate			Average	St Dev
0		0.43		0.43	
15	0.34	0.35	0.00	0.23	0.20
27	0.22	0.39	0.28	0.30	0.09
Day	Lactose 50 g soil			Average	St Dev
0					
15	0.27	0.13	0.35	0.25	0.11
27	0.50	0.24	0.27	0.34	0.14
Day	Autoclaved Control			Average	St Dev
0		3.89		3.89	
15	0.34	0.80	0.29	0.48	0.28
27	0.32	0.46	0.20	0.33	0.13
Day	Control 50 g soil			Average	St Dev
0					
15	0.26	0.35	0.28	0.30	0.05
27	0.07	0.33	0.73	0.38	0.33

During the Run 1 experiment, the DO levels in the control dropped to 0.3 mg/L at 12 days and to 0.2 at 27 days. However, because the standard deviation of both of these measurements was 0.3, it is likely that this change was not significant.

The DO levels of the amended reactors in Run 1 decreased for the 12-day measurement. Except for corn syrup, this decrease would likely have been statistically significant. However, for the 27-day measurement, the DO measurements for corn syrup, EOS, and acetate actually increased compared to the 12-day measurement. The 27-day DO measurements for molasses and lactose continued to decrease.

As mentioned above, unfortunately, the team failed to record a time zero measurement for Run 2, except for the autoclaved control. This measurement was 3.9 mg/L. The subsequent DO measurements for all the reactors were similar levels to that found in Run 1.

Tables 15 and 16 summarize Eh measurements from Runs 1 and 2. Unfortunately, data from time zero was not taken. Again, the variability in the reactors made it difficult to obtain measurements with any reliability. This variability is a common confounding factor in Eh measurements (Vance 1996). Measurements taken from the Run 1 control on days 12 and 27 had Eh's of -52 and -95, respectively. These levels are in the zone of anaerobic reactions, in the range of sulfate reduction (Vance, 1996). Amendments depressed Eh further to levels ranging from -147 to -277, into the range of methane reduction. Measurements of controls in Run 2 were lower, on the same levels of those taken in the amended reactors.

pH

Tables 17 and 18 summarize the pH data for Runs 1 and 2, respectively. The initial measurements were not triplicated; however, subsequent measurements were. For Run 1, the initial pH measurement of the non-autoclaved control was 8.5. The initial measurements for the amended treatments in Run 1 ranged from 7.1 to 8.9. The pH declined slightly (from 0.9 to 1.7 pH units) for each amended treatment over the 27-day run.

During Run 2, the pH's for most of the reactors remained close to the initial measurement level. Two exceptions to this were measurements for Whey and Lactose (50g soil/500 mL solution), which had modest, but noticeable pH declines throughout the 27-day study.

Table 15. Run 1 Eh.

Day	Control			Average	St Dev	mVAgCl	mV ref Eh
0							
12	-49	-50	-56	-52	4	-92	108
27	-71	-92	-122	-95	26	-135	65
Day	Corn Syrup			Average	St Dev	mVAgCl	mV ref Eh
0							
12	-366	-175	-215	-252	101	-292	-92
27	-171	-165	-182	-172	9	-212	-12
Day	Molasses			Average	St Dev	mVAgCl	mV ref Eh
0							
12	-252	-284	-256	-264	17	-304	-104
27	-211	-202	-213	-209	6	-249	-49
Day	Lactose			Average	St Dev	mVAgCl	mV ref Eh
0							
12	-225	-256	-198	-226	29	-266	-66
27	-137	-159	-145	-147	11	-187	13
Day	EOS			Average	St Dev	mVAgCl	mV ref Eh
0							
12	-264	-280	-286	-277	12	-317	-117
27	-174	-156	-176	-169	11	-209	-9
Day	Acetate			Average	St Dev	mVAgCl	mV ref Eh
0							
12	-259	-239	-225	-241	17	-281	-81
27	-197	-210	-254	-220	30	-260	-60

Metals and Conductivity Data

Table 19 and 20 summarize metals and conductivity data from Run 1. For Run 1, it is clear that the metals increased in solution over the 27-day run. Increases were found for Mn in the control. Greater increases were found in the amendment treatments for Mn, Fe, and Ca. Presumably, these metals were leached from the soils that were in the system. Although some leaching appeared to occur in the control, much more was found in the amended reactors. So, either the amendments themselves facilitated leaching or the bioactivity spurred from the amendments resulted in enhanced metals leaching. Bednar et al. (2007) indicated that added organic acids could result in increased uranium solubility. Similarly, bacterial activity can produce organic acids and biological chelating agents that increase

Table 16. Run 2 Eh.

Day	Control			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-373.5	-298.9	-164.2	-279	106	-319	-119
27	-738.8	-322.1	-271.4	-444	256	-484	-284
Day	Benzoic acid			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-176.0	-295.8	-311.5	-261	74	-301	-101
27	-303.3	-411.3	-356.7	-357	54	-397	-197
Day	Ethanol			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-323.3	-277.8	-290.9	-297	23	-337	-137
27	-379.7	-387.8	-379.7	-382	5	-422	-222
Day	Whey			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-207.5	-180.2	-198.9	-196	14	-236	-36
27	-232.8	-210.2	-227.6	-224	12	-264	-64
Day	Na Lactate			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-163.2	-271.4	-111.8	-182	81	-222	-22
27	-259.8	-196.9	-157.5	-205	52	-245	-45
Day	Lactose 50 g soil			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-211.4	-226.4	-238.3	-225	13	-265	-65
27	-187.0	-186.1	-178.9	-184	4	-224	-24
Day	Autoclaved Control			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-106.9	-65.6	-154.9	-109	45	-149	51
27	-160.0	-166.7	-185.6	-171	13	-211	-11
Day	Control 50 g soil			Average	St Dev	mVAgCl	mV ref Eh
0							
15	-172.8	-189.1	-148.6	-170	20	-210	-10
27	-178.6	-143.3	-172.0	-165	19	-205	-5

Table 17. Run 1 pH.

Day	Control			Average	St Dev
0		8.5		8.5	
12	8.4	8.4	8.5	8.4	0.0
27	7.9	8.1	8.2	8.1	0.2
Day	Corn syrup			Average	St Dev
0		8.8		8.8	
12	6.3	6.6	7.2	6.7	0.5
27	6.9	7.1	7.3	7.1	0.2
Day	Molasses			Average	St Dev
0		8.3		8.3	
12	6.3	6.7	6.6	6.6	0.2
27	7.4	7.4	7.5	7.4	0.1
Day	Lactose			Average	St Dev
0		7.4		7.4	
12	5.4	5.7	5.4	5.5	0.2
27	6.0	6.2	6.0	6.1	0.1
Day	EOS			Average	St Dev
0		7.1		7.1	
12	6.6	6.8	6.9	6.8	0.2
27	6.4	6.1	6.4	6.3	0.2
Day	Acetate			Average	St Dev
0		8.9		8.9	
12	8.4	8.3	8.4	8.3	0.1
27	7.4	8.0	8.1	7.8	0.4

metallic solubility. As discussed above, pH decreases were found for some of the more effective amendments, particularly those in Run 1. Conductivity measurements for Run 1 steadily increased as well, supporting the conclusion that metals dissolved into solution over time.

Tables 21 and 22 summarize metals and conductivity data for Run 2. A similar pattern of metals increase in solution was found as in Run 1, although it was not as pronounced. Interestingly, virtually no increase was found in the autoclaved control, suggesting that retarding the biological activity decreased metals dissolution.

Table 18. Run 2 pH.

Day	Control			Average	St Dev
0		8.2		8.2	
15	7.7	7.9	8.0	7.9	0.2
27	8.0	8.2	8.3	8.2	0.2
Day	Benzoic acid			Average	St Dev
0		8.0		8.0	
15	8.1	8.1	8.2	8.1	0.1
27	8.3	8.3	8.4	8.3	0.0
Day	Ethanol			Average	St Dev
0		8.6		8.6	
15	8.3	8.3	8.3	8.3	0.0
27	8.4	8.4	8.5	8.4	0.1
Day	Whey			Average	St Dev
0		8.3		8.3	
15	7.8	7.5	7.7	7.7	0.1
27	8.0	7.7	8.0	7.9	0.2
Day	Na Lactate			Average	St Dev
0		7.6		7.6	
15	8.1	8.1	8.2	8.2	0.0
27	8.0	8.3	8.3	8.2	0.2
Day	Lactose 50 g soil			Average	St Dev
0		7.5		7.5	
15	5.4	5.4	5.4	5.4	0.0
27	5.7	6.7	5.7	6.0	0.6
Day	Autoclaved Control			Average	St Dev
0		7.6		7.6	
15	6.6	7.1	7.3	7.0	0.4
27	8.0	8.0	8.1	8.0	0.0
Day	Control 50 g soil			Average	St Dev
0		7.6		7.6	
15	7.7	8.0	8.1	8.0	0.2
27	8.4	8.7	8.5	8.6	0.1

Table 19. Run 1 Metals Analysis. Detection limit was 0.5 µg/L. BD means below detection.

Date	Fe (µg/L)	Mn (µg/L)	Ca (µg/L)
Control			
0	BD	36	30,293
12	BD	320	32,147
27	BD	244	28,920
Corn Syrup			
0	BD	66	31,330
12	5,903	10,033	340,800
27	3,480	13,060	443,367
Molasses			
0	121	347	43,880
12	2,347	11,443	378,600
27	2,230	11,670	414,200
Lactose			
0	BD	92	62,157
12	20,114	15,637	642,333
27	31,573	23,373	800,067
EOS			
0	BD	38	46,123
12	75	3,784	97,807
27	12,106	12,361	275,133
Acetate			
0	BD	57	60,703
12	BD	1,171	96,872
27	BD	1,018	100,970

Cost and Sources of Amendments

The sources and costs of the amendments with the most promising performance were identified and are summarized in Tables 23 and 24.

Table 20. Run 1 Conductivity $\mu\text{S}/\text{cm}$.

Day	Control			Average	St Dev
0		413		413	
12	325	323	320	323	3
27	332	323	320	325	6
Day	Corn Syrup			Average	St Dev
0		317		317	
12	1,705	1,753	1,420	1,626	180
27	1,703	2,111	2,059	1,958	222
Day	Molasses			Average	St Dev
0		390		390	
12	1,767	1,767	1,789	1,774	13
27	1,793	1,875	1,804	1,824	45
Day	Lactose			Average	St Dev
0		927		927	
12	2,958	2,848	3,540	3,115	372
27	3,600	3,010	4,110	3,573	550
Day	EOS			Average	St Dev
0		534		534	
12	775	731	718	741	30
27	1855	1384	1101	1,447	381
Day	Acetate			Average	St Dev
0		2131		2,131	
12	2027	2090	2060	2,059	32
27	2036	2081	2056	2,058	23

Table 21. Run 2 Metals Analysis. Detection limit was 0.5 µg/L. BD means below detection.

Date	Fe (µg/L)	Mn (µg/L)	Ca (µg/L)
Control			
0	BD	55	37,913
15	108	4	46,013
27	17	253	38,320
Benzoic Acid			
0	BD	26	41,570
15	37	2	46,453
27	BD	436	33,970
Ethanol			
0	BD	12	42,437
15	BD	BD	48,467
27	BD	423	21,970
Whey			
0	BD	21	59,817
15	BD	BD	41,013
27	71	3,467	102,873
Na Lactate			
0	13	68	114,967
15	BD	12	54,300
27	BD	1,807	47,127
Lactose with 50 g soil			
0	BD	39	71,623
15	BD	13	71,897
27	23,880	5,655	105,907
Autoclaved Control			
0	BD	14	46,970
15			
27	3	20	22,238
Control with 50 g soil			
0	BD	16	33,943
15			
27	BD	13	18,843

Table 22. Run 2 Conductivity $\mu\text{S}/\text{cm}$.

Day	Control			Average	St Dev
0		104		104	
15	410	416	410	412	3
27	408	414	411	411	3
Day	Benzoic acid			Average	St Dev
0		121		121	
15	458	467	471	465	7
27	453	466	466	462	8
Day	Ethanol			Average	St Dev
0		133		133	
15	412	412	403	409	5
27	409	409	402	407	4
Day	Whey			Average	St Dev
0		172		172	
15	1638	1389	1160	1396	239
27	1143	1425	1190	1253	151
Day	Na Lactate			Average	St Dev
0		2935		2935	
15	3030	2890	3060	2993	91
27	3040	2914	3060	3005	79
Day	Lactose 50 g soil			Average	St Dev
0		160		160	
15	1910	1973	1970	1951	36
27	2106	2109	2157	2124	29
Day	Autoclaved Control			Average	St Dev
0		377		377	
15	349	369	365	361	11
27		340	375	371	25
Day	Control 50 g soil			Average	St Dev
0		417		417	
15	374	372	371	372	2
27	355	345	348	349	5

Table 23. Amendment costs.

Amendment	Quantity/container	Cost	units
sodium acetate	50 lb bag	1.63	\$/lb
JWR lactate concentrate	606 lb drum	1.20	\$/lb
molasses	645 lb drum	0.45	\$/lb
corn syrup	646 lb drum	0.40	\$/lb
ethanol	55 gal drum	2.50	\$/gal
benzoic acid	55 lb bag	1.642	\$/lb
EOS	55 gal drum	1400	\$/drum
lactose	Tanker	none	Transport
whey	Tanker	0.15	\$/lb

Table 24. Sources for Key Amendments.

Amendment	Supplier	Contact	Phone	Address
Lactose	Columbia River Processing	Roy Dugan	541-481-3770	79588 Rippee Road
Whey	Columbia River Processing	Roy Dugan	541-481-3771	79588 Rippee Road
55 High Fructose Corn Syrup	Malt Products Corp.	Joanne McGuire	530-677-8282	
#677 Blackstrap Molasses	Malt Products Corp.	Joanne McGuire	530-677-8283	
Ethanol	Pacific Ethanol (office)	Sheril Pagard	916 403 2129	400 Capitol Mall
	Pacific Ethanol Columbia LLC	no contact yet	541 481-2716	71335 Rail Loop Dr
Milk Hauler from Boardman	LTI, Inc (Milky Way)	Brad Williamson	800 327 6255	8631 Depot Road
EOS	EOS Remediation	Anne Borden	919.873.2204 ext 111	1101 Nowell Road
	EOS Remediation	Timothy Parker	919.873.2204 ext 173	

4 Recommendations

Our experiments suggest that TNT removal is relatively easy to stimulate. However, RDX removal is more difficult; therefore, it is best to focus on the product that is the most efficient at removing RDX when selecting the best amendment. Based on our results, five amendments stand out for their ability to remove RDX:

- Molasses
- Corn syrup
- EOS
- Lactose
- Whey

Lactose and whey are both dairy by-products. The lactose product is, essentially, the filtered product of whey. As such, it has much lower solid materials than whey. The particulate material in the whey may cause clogging issues during injection. Furthermore, lactose is available for only the shipping costs. Therefore, lactose was chosen as the substrate to test for the push-pull test.

We also compared corn syrup, molasses, and EOS. All performed well in our testing, but EOS has the advantage of being a longer-lasting material. Although the ethanol treatment was not particularly effective in these studies, discussions among members of the Umatilla Bioremediation Team led to the conclusion that ethanol does have key advantages. In particular, it is quite stable and therefore could be employed in automatic injection systems with minimal threat of degradation over time. In addition, ethanol is known to be effective at reducing well clogging. The study found some evidence of a delayed degradation for ethanol; other studies have found that ethanol can be an effective co-substrate. Thus it was concluded that ethanol should be studied in the push-pull test.

This report should be cited as follows:

Medina, V.F., H. Knotek-Smith, D. Gent, and A. Morrow. 2010. *The results of a laboratory feasibility study for the biological treatment of Umatilla Groundwater*. ERDC/EL TR-11-xx. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

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Appendix A: Raw Explosive Analysis Data

Table A1. Run 1 TNT Raw Data.

TNT	24-Feb-10	25-Feb-10	26-Feb-10	1-Mar-10	4-Mar-10	8-Mar-10	11-Mar-10	16-Mar-10	23-Mar-10
1	1.050	0.785	0.550	0.248	0.155	0.000	0.000	0.000	0.000
2	1.010	0.777	0.538	0.237	0.154	0.000	0.000	0.000	0.000
3	1.000	0.875	0.704	0.241	0.136	0.000	0.000	0.000	0.000
TNT Control	1.020	0.812	0.597	0.242	0.148	0.000	0.000	0.000	0.000
St Dev	0.022	0.044	0.076	0.005	0.009	0.000	0.000	0.000	0.000
4	1.000	0.679	0.426	0.000	0.000	0.000	0.000	0.000	0.000
5	1.030	0.708	0.445	0.000	0.000	0.000	0.000	0.000	0.000
6	1.060	0.709	0.540	0.000	0.000	0.000	0.000	0.000	0.000
TNT Corn Syrup	1.030	0.699	0.470	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.024	0.014	0.050	0.000	0.000	0.000	0.000	0.000	0.000
7	1.050	0.493	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	1.030	0.447	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	1.030	0.420	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TNT Molasses	1.037	0.453	0.000	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.009	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	1.060	0.176	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11	1.080	0.149	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12	1.080	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TNT Lactose	1.073	0.164	0.000	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.009	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13	0.602	0.412	0.204	0.000	0.000	0.000	0.000	0.000	0.000
14	0.552	0.386	0.174	0.000	0.000	0.000	0.000	0.000	0.000
15	0.553	0.397	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TNT EOS	0.569	0.398	0.126	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.023	0.011	0.090	0.000	0.000	0.000	0.000	0.000	0.000
16	1.100	0.781	0.581	0.259	0.146	0.000	0.000	0.000	0.000
17	1.130	0.801	0.579	0.211	0.000	0.000	0.000	0.000	0.000
18	1.060	0.727	0.520	0.195	0.000	0.000	0.000	0.000	0.000
TNT Acetate	1.097	0.770	0.560	0.222	0.049	0.000	0.000	0.000	0.000
St Dev	0.029	0.031	0.028	0.027	0.069	0.000	0.000	0.000	0.000

Table A2. Run 1 4 Amino 2,6-dinitroitoluene Raw Data.

	24-Feb-10	25-Feb-10	26-Feb-10	1-Mar-10	4-Mar-10	8-Mar-10	11-Mar-10	16-Mar-10	23-Mar-10
1	0.000	0.000	0.027	0.098	0.115	0.136	0.117	0.000	0.000
2	0.000	0.000	0.035	0.103	0.118	0.138	0.131	0.078	0.003
3	0.000	0.000	0.052	0.089	0.124	0.143	0.086	0.000	0.000
4-A-2,6-D Control	0.000	0.000	0.038	0.097	0.119	0.139	0.111	0.026	0.001
St Dev	0.000	0.000	0.010	0.006	0.004	0.003	0.019	0.037	0.001
4	0.000	0.025	0.075	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.018	0.054	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.022	0.085	0.117	0.000	0.000	0.000	0.000	0.000
4-A-2,6-D Corn Syrup	0.000	0.022	0.071	0.039	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.003	0.013	0.055	0.000	0.000	0.000	0.000	0.000
7	0.000	0.081	0.164	0.000	0.000	0.000	0.000	0.000	0.000
8	0.000	0.092	0.182	0.000	0.000	0.000	0.000	0.000	0.000
9	0.000	0.084	0.162	0.000	0.000	0.000	0.000	0.000	0.000
4-A-2,6-D Molasses	0.000	0.086	0.169	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
10	0.000	0.176	0.372	0.000	0.000	0.000	0.000	0.000	0.000
11	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12	0.000	0.193	0.375	0.000	0.000	0.000	0.000	0.000	0.000
4-A-2,6-D Lactose	0.000	0.190	0.249	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.010	0.176	0.000	0.000	0.000	0.000	0.000	0.000
13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000
4-A-2,6-D EOS	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
16	0.000	0.000	0.040	0.000	0.126	0.143	0.107	0.000	0.000
17	0.000	0.000	0.041	0.000	0.136	0.146	0.129	0.037	0.000
18	0.000	0.000	0.049	0.000	0.140	0.143	0.109	0.044	0.000
4-A-2,6-D Acetate	0.000	0.000	0.043	0.000	0.134	0.144	0.115	0.027	0.000
St Dev	0.000	0.000	0.004	0.000	0.006	0.001	0.010	0.019	0.000

Table A3. Run 1 2 Amino 4,6 dinitrotoluene Raw Data.

	24-Feb-10	25-Feb-10	26-Feb-10	1-Mar-10	4-Mar-10	8-Mar-10	11-Mar-10	16-Mar-10	23-Mar-10
1	0.000	0.000	0.016	0.034	0.037	0.029	0.000	0.000	0.000
2	0.000	0.000	0.018	0.032	0.045	0.048	0.030	0.000	0.000
3	0.000	0.000	0.025	0.030	0.042	0.028	0.000	0.000	0.000
2-A-4,6-D Control	0.000	0.000	0.020	0.032	0.041	0.035	0.010	0.000	0.000
St Dev	0.000	0.000	0.004	0.002	0.003	0.009	0.014	0.000	0.000
4	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
2-A-4,6-D Corn Syrup	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.049	0.000	0.000	0.000	0.000	0.000	0.000
9	0.000	0.010	0.051	0.000	0.000	0.000	0.000	0.000	0.000
2-A-4,6-D Molasses	0.000	0.003	0.046	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000
10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2-A-4,6-D Lactose	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13	0.000	0.000	0.204	0.000	0.000	0.000	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2-A-4,6-D EOS	0.000	0.000	0.068	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.096	0.000	0.000	0.000	0.000	0.000	0.000
16	0.000	0.000	0.019	0.062	0.044	0.030	0.000	0.000	0.000
17	0.000	0.000	0.019	0.070	0.038	0.015	0.000	0.000	0.000
18	0.000	0.000	0.018	0.065	0.052	0.181	0.000	0.000	0.000
2-A-4,6-D Acetate	0.000	0.000	0.019	0.066	0.045	0.075	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.003	0.006	0.075	0.000	0.000	0.000

Table A4. Run 1 HMX Raw Data.

HMX	24-Feb-10	25-Feb-10	26-Feb-10	1-Mar-10	4-Mar-10	8-Mar-10	11-Mar-10	16-Mar-10	23-Mar-10
1	0.671	0.950	1.200	1.550	1.530	1.730	1.790	1.430	1.450
2	0.636	0.919	1.100	1.510	1.540	1.650	1.740	1.590	1.460
3	0.678	1.040	1.310	1.470	1.480	1.620	1.670	1.270	1.330
HMX Control	0.662	0.970	1.203	1.510	1.517	1.667	1.733	1.430	1.413
St Dev	0.018	0.051	0.086	0.033	0.026	0.046	0.049	0.131	0.059
4	0.592	0.841	0.743	1.480	1.440	0.998	0.727	0.056	0.092
5	0.610	0.852	0.837	1.520	1.350	0.829	0.469	0.225	0.000
6	0.685	0.879	1.010	1.490	1.150	1.200	1.300	0.750	0.000
HMX Corn Syrup	0.629	0.857	0.863	1.497	1.313	1.009	0.832	0.344	0.031
St Dev	0.040	0.016	0.111	0.017	0.121	0.152	0.347	0.295	0.043
7	0.411	0.839	1.180	1.510	1.380	1.140	0.904	0.343	0.000
8	0.396	0.837	1.310	1.650	1.630	1.350	1.110	0.404	0.000
9	0.447	0.776	0.950	1.480	1.360	1.180	1.080	0.364	0.000
HMX Molasses	0.418	0.817	1.147	1.547	1.457	1.223	1.031	0.370	0.000
St Dev	0.021	0.029	0.149	0.074	0.123	0.091	0.091	0.025	0.000
10	0.426	0.980	1.500	1.670	1.460	1.330	1.330	1.300	1.090
11	0.390	0.820	1.370	1.180	1.220	1.140	1.120	0.687	0.376
12	0.397	0.962	1.490	1.620	1.090	1.090	1.100	0.908	0.873
HMX Lactose	0.404	0.921	1.453	1.490	1.257	1.187	1.183	0.965	0.780
St Dev	0.016	0.072	0.059	0.220	0.153	0.103	0.104	0.253	0.299
13	0.360	0.722	1.280	1.350	1.420	1.310	1.140	0.803	0.708
14	0.382	0.678	1.150	1.290	1.210	1.200	1.130	0.764	0.584
15	0.046	0.796	1.260	1.360	1.300	1.300	1.230	0.838	0.744
HMX EOS	0.263	0.732	1.230	1.333	1.310	1.270	1.167	0.802	0.679
St Dev	0.153	0.049	0.057	0.031	0.086	0.050	0.045	0.030	0.069
16	0.424	0.868	1.300	1.530	1.600	1.700	1.910	1.370	1.390
17	0.441	0.960	1.430	1.670	1.720	1.890	1.930	1.660	1.490
18	0.401	0.804	1.190	1.340	1.370	1.490	1.530	1.110	1.200
HMX Acetate	0.422	0.877	1.307	1.513	1.563	1.693	1.790	1.380	1.360
St Dev	0.016	0.064	0.098	0.135	0.145	0.163	0.184	0.225	0.120

Table A5. Run 1 RDX Raw Data.

RDX	24-Feb-10	25-Feb-10	26-Feb-10	1-Mar-10	4-Mar-10	8-Mar-10	11-Mar-10	16-Mar-10	23-Mar-10
1	0.787	0.786	0.808	0.819	0.743	0.760	0.740	0.588	0.554
2	0.742	0.768	0.784	0.784	0.749	0.759	0.769	0.679	0.612
3	0.733	0.867	1.140	0.766	0.726	0.718	0.704	0.482	0.511
RDX Control	0.754	0.807	0.911	0.790	0.739	0.746	0.738	0.583	0.559
St Dev	0.024	0.043	0.162	0.022	0.010	0.020	0.027	0.081	0.041
4	0.757	0.767	0.808	0.093	0.000	0.000	0.000	0.000	0.000
5	0.776	0.774	0.775	0.078	0.000	0.000	0.000	0.000	0.000
6	0.804	0.780	0.998	0.723	0.437	0.133	0.000	0.000	0.000
RDX Corn Syrup	0.779	0.774	0.860	0.298	0.146	0.044	0.000	0.000	0.000
St Dev	0.019	0.005	0.098	0.301	0.206	0.063	0.000	0.000	0.000
7	0.765	0.737	0.690	0.529	0.000	0.000	0.000	0.000	0.000
8	0.754	0.778	0.754	0.482	0.067	0.000	0.000	0.000	0.000
9	0.774	0.735	0.653	0.463	0.000	0.000	0.000	0.000	0.000
RDX Molasses	0.764	0.750	0.699	0.491	0.022	0.000	0.000	0.000	0.000
St Dev	0.008	0.020	0.042	0.028	0.032	0.000	0.000	0.000	0.000
10	0.756	0.732	0.678	0.227	0.000	0.000	0.000	0.000	0.000
11	0.752	0.678	0.654	0.000	0.000	0.000	0.000	0.000	0.000
12	0.763	0.726	0.662	0.025	0.000	0.000	0.000	0.000	0.000
RDX Lactose	0.757	0.712	0.665	0.084	0.000	0.000	0.000	0.000	0.000
St Dev	0.005	0.024	0.010	0.102	0.000	0.000	0.000	0.000	0.000
13	0.717	0.762	0.747	0.000	0.000	0.000	0.000	0.000	0.000
14	0.751	0.742	0.758	0.000	0.000	0.000	0.000	0.000	0.000
15	0.741	0.744	0.759	0.000	0.000	0.000	0.000	0.000	0.000
RDX EOS	0.736	0.749	0.755	0.000	0.000	0.000	0.000	0.000	0.000
St Dev	0.014	0.009	0.005	0.000	0.000	0.000	0.000	0.000	0.000
16	0.773	0.752	0.784	0.792	0.785	0.756	0.752	0.645	0.518
17	0.788	0.782	0.804	0.804	0.782	0.764	0.761	0.727	0.538
18	0.776	0.761	0.778	0.772	0.753	0.721	0.708	0.585	0.500
RDX Acetate	0.779	0.765	0.789	0.789	0.773	0.747	0.740	0.652	0.519
St Dev	0.006	0.013	0.011	0.013	0.014	0.019	0.023	0.058	0.016

Table A6. Run 1 MNX Raw Data.

[illegible]

Table A8. Run 1 TNX Raw Data.

[illegible]

Table A9. Run 2 TNT Raw Data.

TNT	0	2	7	15	24
1	1.010	0.674	0.201	0.000	0.000
2	0.994	0.651	0.206	0.000	0.000
3	1.010	0.629	0.175	0.000	0.000
TNT Control	1.005	0.651	0.194	0.000	0.000
St Dev	0.008	0.018	0.014	0.000	0.000
4	1.020	0.826	0.326	0.000	0.000
5	1.010	0.827	0.340	0.000	0.000
6	1.030	0.823	0.344	0.000	0.000
TNT Benzoic Acid	1.020	0.825	0.337	0.000	0.000
St Dev	0.008	0.002	0.008	0.000	0.000
7	1.010	0.664	0.191	0.000	0.000
8	0.980	0.635	0.180	0.000	0.000
9	1.260	0.571	0.168	0.000	0.000
TNT Ethanol	1.083	0.623	0.180	0.000	0.000
St Dev	0.126	0.039	0.009	0.000	0.000
10	0.994	0.000	0.000	0.000	0.000
11	0.983	0.000	0.000	0.000	0.000
12	1.010	0.000	0.000	0.000	0.000
TNT Whey	0.996	0.000	0.000	0.000	0.000
St Dev	0.011	0.000	0.000	0.000	0.000
13	1.040	0.759	0.199	0.000	0.000
14	1.010	0.699	0.168	0.000	0.000
15	1.020	0.718	0.202	0.000	0.000
TNT Na Lactate	1.023	0.725	0.190	0.000	0.000
St Dev	0.012	0.025	0.015	0.000	0.000
16	1.070	0.000	0.000	0.000	0.000
17	1.050	0.000	0.000	0.000	0.000
18	1.070	0.000	0.000	0.000	0.000
TNT L. Soil Lactose	1.063	0.000	0.000	0.000	0.000
St Dev	0.009	0.000	0.000	0.000	0.000
19	1.120	1.030	0.542	0.471	0.097
20	1.130	1.060	0.513	0.343	0.103
21	1.180	0.663	0.568	0.431	0.209
TNT Dead Control	1.143	0.918	0.541	0.415	0.136
St Dev	0.026	0.180	0.022	0.053	0.051
22	1.120	0.927	0.748	0.647	0.265
23	1.090	0.964	0.779	0.745	0.285
24	1.150	0.824	0.772	0.778	0.369
TNT L. Soil Control	1.120	0.905	0.766	0.723	0.306
St Dev	0.024	0.059	0.013	0.056	0.045

Table A10. Run 2 4 Amino 2,6-dinitroitoluene Raw Data.

4 Amino 2,6-dinitroitoluene	0	2	7	15	24
1	0.000	0.022	0.038	0.070	0.038
2	0.000	0.026	0.055	0.153	0.061
3	0.000	0.033	0.058	0.154	0.077
4A26D Control	0.000	0.027	0.050	0.126	0.059
St Dev	0.000	0.005	0.009	0.039	0.016
4	0.000	0.000	0.027	0.139	0.143
5	0.000	0.010	0.066	0.181	0.195
6	0.000	0.000	0.034	0.165	0.155
4A26D Benzoic Acid	0.000	0.003	0.042	0.162	0.164
St Dev	0.000	0.005	0.017	0.017	0.022
7	0.000	0.019	0.056	0.165	0.041
8	0.000	0.019	0.052	0.122	0.000
9	0.000	0.030	0.080	0.147	0.067
4A26D Ethanol	0.000	0.023	0.063	0.145	0.036
St Dev	0.000	0.005	0.012	0.018	0.028
10	0.000	0.000	0.000	0.216	0.000
11	0.000	0.241	0.000	0.315	0.000
12	0.000	0.000	0.000	0.000	0.000
4A26D Whey	0.000	0.080	0.000	0.177	0.000
St Dev	0.000	0.114	0.000	0.132	0.000
13	0.000	0.018	0.066	0.100	0.000
14	0.000	0.020	0.080	0.000	0.000
15	0.000	0.013	0.064	0.000	0.000
4A26D Na Lactate	0.000	0.017	0.070	0.033	0.000
St Dev	0.000	0.003	0.007	0.047	0.000
16	0.000	0.000	0.000	0.011	0.000
17	0.000	0.000	0.000	0.033	0.000
18	0.000	0.000	0.000	0.032	0.000
4A26D L. Soil Lactose	0.000	0.000	0.000	0.025	0.000
St Dev	0.000	0.000	0.000	0.010	0.000
19	0.000	0.000	0.036	0.048	0.068
20	0.000	0.000	0.025	0.059	0.060
21	0.000	0.000	0.044	0.067	0.058
4A26D Dead Control	0.000	0.000	0.035	0.058	0.062
St Dev	0.000	0.000	0.008	0.008	0.004
22	0.000	0.000	0.000	0.010	0.037
23	0.000	0.000	0.000	0.011	0.043
24	0.000	0.000	0.000	0.011	0.051
4A26D L. Soil Control	0.000	0.000	0.000	0.011	0.044
St Dev	0.000	0.000	0.000	0.000	0.006

Table A11. Run 2 2 Amino 4,6 dinitrotoluene Raw Data.

2 Amino 4,6 dinitrotoluene	0	2	7	15	24
1	0.000	0.000	0.014	0.000	0.000
2	0.000	0.000	0.015	0.000	0.000
3	0.000	0.000	0.023	0.030	0.000
2A46D Control	0.000	0.000	0.017	0.010	0.000
St Dev	0.000	0.000	0.004	0.014	0.000
4	0.000	0.000	0.012	0.035	0.000
5	0.000	0.000	0.048	0.049	0.000
6	0.000	0.000	0.015	0.038	0.000
2A46D Benzoic Acid	0.000	0.000	0.025	0.041	0.000
St Dev	0.000	0.000	0.016	0.006	0.000
7	0.000	0.000	0.000	0.010	0.000
8	0.000	0.000	0.010	0.000	0.000
9	0.000	0.000	0.029	0.020	0.000
2A46D Ethanol	0.000	0.000	0.013	0.010	0.000
St Dev	0.000	0.000	0.012	0.008	0.000
10	0.000	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000	0.000
12	0.000	0.000	0.000	0.000	0.000
2A46D Whey	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
13	0.000	0.000	0.016	0.000	0.000
14	0.000	0.000	0.016	0.000	0.000
15	0.000	0.000	0.014	0.000	0.000
2A46D Na Lactate	0.000	0.000	0.015	0.000	0.000
St Dev	0.000	0.000	0.001	0.000	0.000
16	0.000	0.000	0.000	0.000	0.000
17	0.000	0.000	0.000	0.000	0.000
18	0.000	0.000	0.000	0.000	0.000
2A46D L. Soil Lactose	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
19	0.000	0.000	0.010	0.015	0.038
20	0.000	0.000	0.000	0.025	0.049
21	0.000	0.000	0.018	0.015	0.040
2A46D Dead Control	0.000	0.000	0.009	0.018	0.042
St Dev	0.000	0.000	0.007	0.005	0.005
22	0.000	0.000	0.000	0.000	0.017
23	0.000	0.000	0.000	0.000	0.021
24	0.000	0.000	0.000	0.000	0.027
2A46D L. Soil Control	0.000	0.000	0.000	0.000	0.022
St Dev	0.000	0.000	0.000	0.000	0.004

Table A12. Run 2 HMX Raw Data.

HMX	0	2	7	15	24
1	0.659	1.200	1.420	1.430	1.240
2	0.666	1.110	1.270	1.380	1.170
3	0.633	1.150	1.340	1.370	1.220
HMX Control	0.653	1.153	1.343	1.393	1.210
St Dev	0.014	0.037	0.061	0.026	0.029
4	0.547	1.100	4.640	1.330	1.230
5	0.685	1.230	4.430	1.490	1.400
6	0.624	1.120	4.220	1.380	1.210
HMX Benzoic Acid	0.619	1.150	4.430	1.400	1.280
St Dev	0.056	0.057	0.171	0.067	0.085
7	0.548	1.150	1.370	1.380	1.220
8	0.523	1.110	1.380	1.470	0.000
9	0.645	1.040	1.350	1.390	1.070
HMX Ethanol	0.572	1.100	1.367	1.413	0.763
St Dev	0.053	0.045	0.012	0.040	0.543
10	0.466	1.140	1.040	0.000	0.000
11	0.427	0.970	1.680	0.076	0.000
12	0.466	1.550	1.130	0.000	0.000
HMX Whey	0.453	1.220	1.283	0.025	0.000
St Dev	0.018	0.243	0.283	0.036	0.000
13	0.494	1.010	1.330	1.330	1.320
14	0.617	1.060	1.340	1.460	1.020
15	0.504	1.030	1.320	1.370	0.914
HMX Na Lactate	0.538	1.033	1.330	1.387	1.085
St Dev	0.056	0.021	0.008	0.054	0.172
16	0.127	0.462	0.212	0.348	0.273
17	0.165	0.430	0.271	0.358	0.257
18	0.154	0.447	0.220	0.374	0.245
HMX L. Soil Lactose	0.149	0.446	0.234	0.360	0.258
St Dev	0.016	0.013	0.026	0.011	0.011
19	0.728	0.800	0.815	0.794	0.678
20	0.729	0.787	0.734	0.761	0.673
21	0.746	0.539	0.946	0.944	0.795
HMX Dead Control	0.734	0.709	0.832	0.833	0.715
St Dev	0.008	0.120	0.087	0.080	0.056
22	0.155	0.275	0.354	0.311	0.287
23	0.147	0.323	0.403	0.339	0.343
24	0.108	0.233	0.344	0.282	0.319
HMX L. Soil Control	0.137	0.277	0.367	0.311	0.316
St Dev	0.021	0.037	0.026	0.023	0.023

Table A13. Run 2 RDX Raw Data.

RDX	0	2	7	15	24
1	0.826	0.830	0.753	0.798	0.622
2	0.800	0.801	0.745	0.759	0.629
3	0.819	0.808	0.756	0.760	0.641
RDX Control	0.815	0.813	0.751	0.772	0.631
St Dev	0.011	0.012	0.005	0.018	0.008
4	0.791	0.788	0.754	0.820	0.718
5	0.810	0.828	0.742	0.833	0.726
6	0.816	0.793	0.767	0.775	0.646
RDX Benzoic Acid	0.806	0.803	0.754	0.809	0.697
St Dev	0.011	0.018	0.010	0.025	0.036
7	0.805	0.798	0.719	0.775	0.621
8	0.772	0.796	0.739	0.769	0.000
9	1.020	0.753	0.728	0.723	0.539
RDX Ethanol	0.866	0.782	0.729	0.756	0.387
St Dev	0.110	0.021	0.008	0.023	0.275
10	0.761	0.492	0.000	0.000	0.000
11	0.757	0.754	0.000	0.026	0.000
12	0.773	0.172	0.000	0.000	0.000
RDX Whey	0.764	0.473	0.000	0.009	0.000
St Dev	0.007	0.238	0.000	0.012	0.000
13	0.792	0.801	0.763	0.775	0.440
14	0.777	0.773	0.727	0.681	0.263
15	0.794	0.796	0.762	0.729	0.205
RDX Na Lactate	0.788	0.790	0.751	0.728	0.303
St Dev	0.008	0.012	0.017	0.038	0.100
16	0.725	0.123	0.000	0.000	0.000
17	0.723	0.016	0.000	0.000	0.000
18	0.763	0.079	0.000	0.000	0.000
RDX L. Soil Lactose	0.737	0.073	0.000	0.000	0.000
St Dev	0.018	0.044	0.000	0.000	0.000
19	0.643	0.620	0.722	0.541	0.468
20	0.637	0.638	0.653	0.545	0.508
21	0.680	0.402	0.705	0.550	0.486
RDX Dead Control	0.653	0.553	0.693	0.545	0.487
St Dev	0.019	0.107	0.029	0.004	0.016
22	0.631	0.554	0.575	0.585	0.479
23	0.620	0.572	0.598	0.603	0.518
24	0.618	0.478	0.567	0.562	0.530
RDX L. Soil Control	0.623	0.535	0.580	0.583	0.509
St Dev	0.006	0.041	0.013	0.017	0.022

Table A14. Run 2 MNX Raw Data.

MNX	0	2	7	15	24
1	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000
MNX Control	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.008
MNX Benzoic Acid	0.000	0.000	0.000	0.000	0.003
St Dev	0.000	0.000	0.000	0.000	0.004
7	0.000	0.000	0.000	0.000	0.028
8	0.000	0.000	0.000	0.066	0.000
9	0.000	0.000	0.000	0.057	0.000
MNX Ethanol	0.000	0.000	0.000	0.041	0.009
St Dev	0.000	0.000	0.000	0.029	0.013
10	0.000	0.223	0.106	0.000	0.000
11	0.000	0.000	0.133	0.000	0.000
12	0.000	0.037	0.116	0.000	0.000
MNX Whey	0.000	0.087	0.118	0.000	0.000
St Dev	0.000	0.098	0.011	0.000	0.000
13	0.000	0.000	0.000	0.000	0.102
14	0.000	0.000	0.000	0.073	0.046
15	0.000	0.000	0.000	0.065	0.055
MNX Na Lactate	0.000	0.000	0.000	0.046	0.068
St Dev	0.000	0.000	0.000	0.033	0.025
16	0.000	0.384	0.086	0.095	0.000
17	0.000	0.330	0.105	0.106	0.000
18	0.000	0.381	0.086	0.097	0.000
MNX L. Soil Lactose	0.000	0.365	0.092	0.099	0.000
St Dev	0.000	0.025	0.009	0.005	0.000
19	0.000	0.000	0.000	0.000	0.000
20	0.000	0.000	0.000	0.000	0.000
21	0.000	0.000	0.000	0.000	0.000
MNX Dead Control	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000	0.000
MNX L. Soil Control	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000

Table A15. Run 2 DNX Raw Data.

DNX	0	2	7	15	24
1	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000
DNX Control	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000
DNX Benzoic Acid	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.070	0.000	0.000
8	0.000	0.000	0.076	0.000	0.000
9	0.000	0.000	0.074	0.000	0.000
DNX Ethanol	0.000	0.000	0.073	0.000	0.000
St Dev	0.000	0.000	0.002	0.000	0.000
10	0.000	0.000	0.293	0.000	0.000
11	0.000	0.000	0.239	0.000	0.000
12	0.000	0.118	0.345	0.000	0.000
DNX Whey	0.000	0.039	0.292	0.000	0.000
St Dev	0.000	0.056	0.043	0.000	0.000
13	0.000	0.000	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000
15	0.000	0.000	0.000	0.000	0.000
DNX Na Lactate	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
16	0.000	0.141	0.175	0.348	0.000
17	0.000	0.266	0.213	0.139	0.000
18	0.000	0.200	0.140	0.117	0.000
DNX L. Soil Lactose	0.000	0.202	0.176	0.201	0.000
St Dev	0.000	0.051	0.030	0.104	0.000
19	0.061	0.000	0.071	0.000	0.000
20	0.000	0.088	0.000	0.000	0.000
21	0.000	0.000	0.000	0.000	0.000
DNX Dead Control	0.020	0.029	0.024	0.000	0.000
St Dev	0.029	0.041	0.033	0.000	0.000
22	0.000	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000	0.000
24	0.000	0.000	0.070	0.000	0.000
DNX L. Soil Control	0.000	0.000	0.023	0.000	0.000
St Dev	0.000	0.000	0.033	0.000	0.000

Table A16. Run 2 TNX Raw Data.

TNX	0	2	7	15	24
1	0.000	0.000	0.000	0.107	0.000
2	0.000	0.000	0.000	0.171	0.000
3	0.000	0.000	0.000	0.000	0.000
TNX Control	0.000	0.000	0.000	0.093	0.000
St Dev	0.000	0.000	0.000	0.071	0.000
4	0.000	0.000	0.000	0.072	0.000
5	0.000	0.000	0.000	0.071	0.000
6	0.000	0.000	0.000	0.072	0.000
TNX Benzoic Acid	0.000	0.000	0.000	0.072	0.000
St Dev	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.000	0.111	0.000
8	0.000	0.000	0.000	0.156	0.000
9	0.000	0.000	0.000	0.139	0.000
TNX Ethanol	0.000	0.000	0.000	0.135	0.000
St Dev	0.000	0.000	0.000	0.019	0.000
10	0.000	0.000	0.076	0.000	0.000
11	0.000	0.000	0.000	0.127	0.000
12	0.000	0.000	0.075	0.000	0.000
TNX Whey	0.000	0.000	0.050	0.042	0.000
St Dev	0.000	0.000	0.036	0.060	0.000
13	0.000	0.000	0.000	0.105	0.000
14	0.000	0.000	0.000	0.211	0.000
15	0.000	0.000	0.000	0.166	0.000
TNX Na Lactate	0.000	0.000	0.000	0.161	0.000
St Dev	0.000	0.000	0.000	0.043	0.000
16	0.000	0.000	0.185	0.243	0.119
17	0.000	0.071	0.184	0.224	0.059
18	0.000	0.062	0.149	0.215	0.055
TNX L. Soil Lactose	0.000	0.044	0.173	0.227	0.078
St Dev	0.000	0.032	0.017	0.012	0.029
19	0.000	0.000	0.000	0.094	0.000
20	0.068	0.000	0.000	0.103	0.000
21	0.066	0.000	0.000	0.104	0.000
TNX Dead Control	0.045	0.000	0.000	0.100	0.000
St Dev	0.032	0.000	0.000	0.004	0.000
22	0.000	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000	0.000
TNX L. Soil Control	0.000	0.000	0.000	0.000	0.000
St Dev	0.000	0.000	0.000	0.000	0.000

Appendix B: Kinetic Analysis of Bioremediation Data

Table B1. TNT Run 1.

TNT	Day	TNT Control	TNT Corn Syrup	TNT Molasses	TNT Lactose	TNT EOS	TNT Acetate
24-Feb-10	0	1.020	1.030	1.037	1.073	0.569	1.097
25-Feb-10	1	0.812	0.699	0.453	0.164	0.398	0.770
26-Feb-10	2	0.597	0.470	0.000	0.000	0.126	0.560
1-Mar-10	5	0.242	0.000	0.000	0.000	0.000	0.222
4-Mar-10	8	0.148	0.000	0.000	0.000	0.000	0.049
8-Mar-10	12	0.000	0.000	0.000	0.000	0.000	0.000
11-Mar-10	15	0.000	0.000	0.000	0.000	0.000	0.000
16-Mar-10	20	0.000	0.000	0.000	0.000	0.000	0.000
23-Mar-10	27	0.000	0.000	0.000	0.000	0.000	0.000
In (Co/C)	Day	Control	Corn Syrup	Molasses	Lactose	EOS	Acetate
	0	0.000	0.000	0.000	0.000	0.000	0.000
	1	0.228	0.388	0.827	1.879	0.357	0.354
	2	0.535	0.784			1.508	0.672
	5	1.439					1.599
	8	1.928					3.115
	12						
	15						
	20						
	27						
k		0.254	0.391	0.827	1.879	0.674	0.368
r ²		0.985	1.000	1.000	1.000	0.898	0.985
t(.5)[=]days		2.725	1.772	0.838	0.369	1.028	1.883

Table B2. RDX Run 1.

RDX	Day	RDX Control	RDX Corn Syrup	RDX Molasses	RDX Lactose	RDX EOS	RDX Acetate
24-Feb-10	0	0.754	0.779	0.764	0.757	0.736	0.779
25-Feb-10	1	0.807	0.774	0.750	0.712	0.749	0.765
26-Feb-10	2	0.911	0.860	0.699	0.665	0.755	0.789
1-Mar-10	5	0.790	0.298	0.491	0.084	0.002	0.789
4-Mar-10	8	0.739	0.146	0.022	0.000	0.000	0.773
8-Mar-10	12	0.746	0.044	0.000	0.000	0.000	0.747
11-Mar-10	15	0.738	0.000	0.000	0.000	0.000	0.740
16-Mar-10	20	0.583	0.000	0.000	0.000	0.000	0.652
23-Mar-10	27	0.559	0.000	0.000	0.000	0.000	0.519
In (Co/C)	Day	Control	Corn Syrup	Molasses	Lactose	EOS	Acetate
	0	0.000	0.000	0.000	0.000	0.000	0.000
	1	-0.068	0.007	0.019	0.061	-0.018	0.018
	2	-0.189	-0.099	0.089	0.130	-0.025	-0.012
	5	-0.046	0.961	0.442	2.199	5.909	-0.013
	8	0.020	1.677	3.533			0.007
	12	0.011	2.866				0.042
	15	0.022					0.051
	20	0.257					0.178
	27	0.299					0.407
k		0.008	0.220	0.326	0.377	0.983	0.010
r ²		0.522	0.942	0.714	0.829	0.774	0.699
t(.5)[=]days		83.512	3.146	2.124	1.838	0.705	70.015

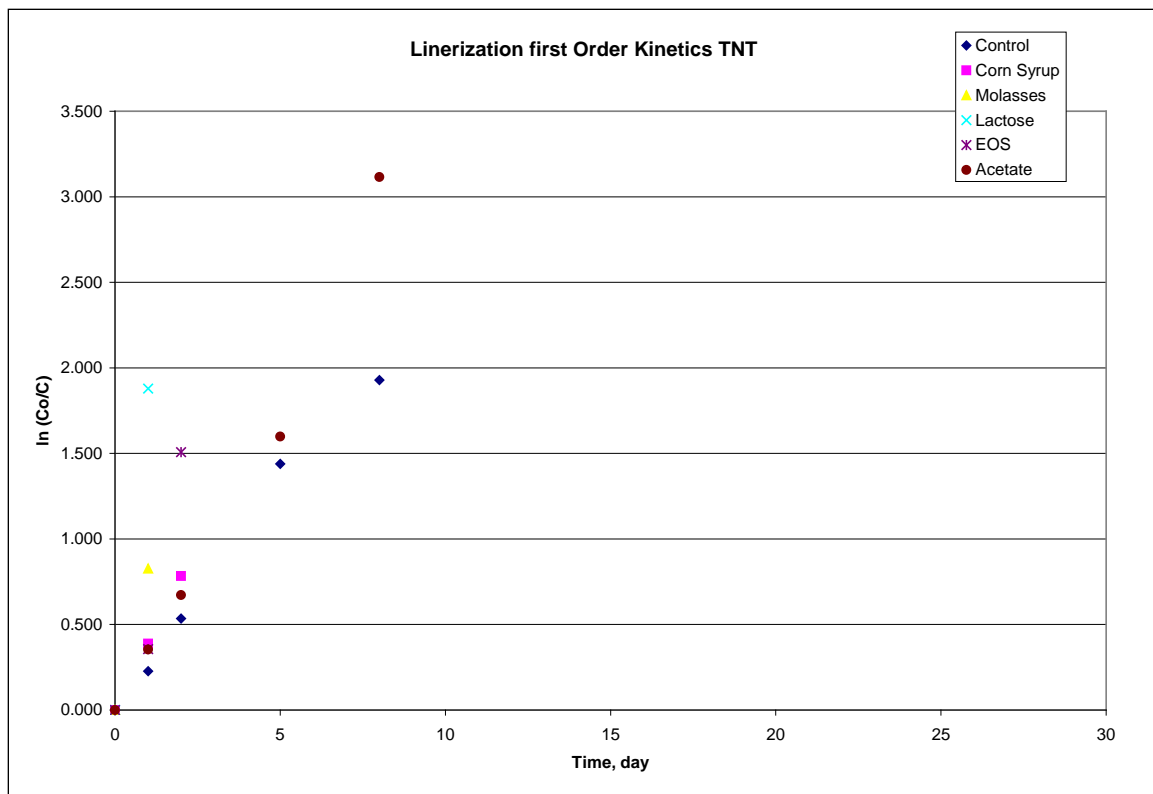


Figure B1. Plot of first-order function linearization TNT Run 1.

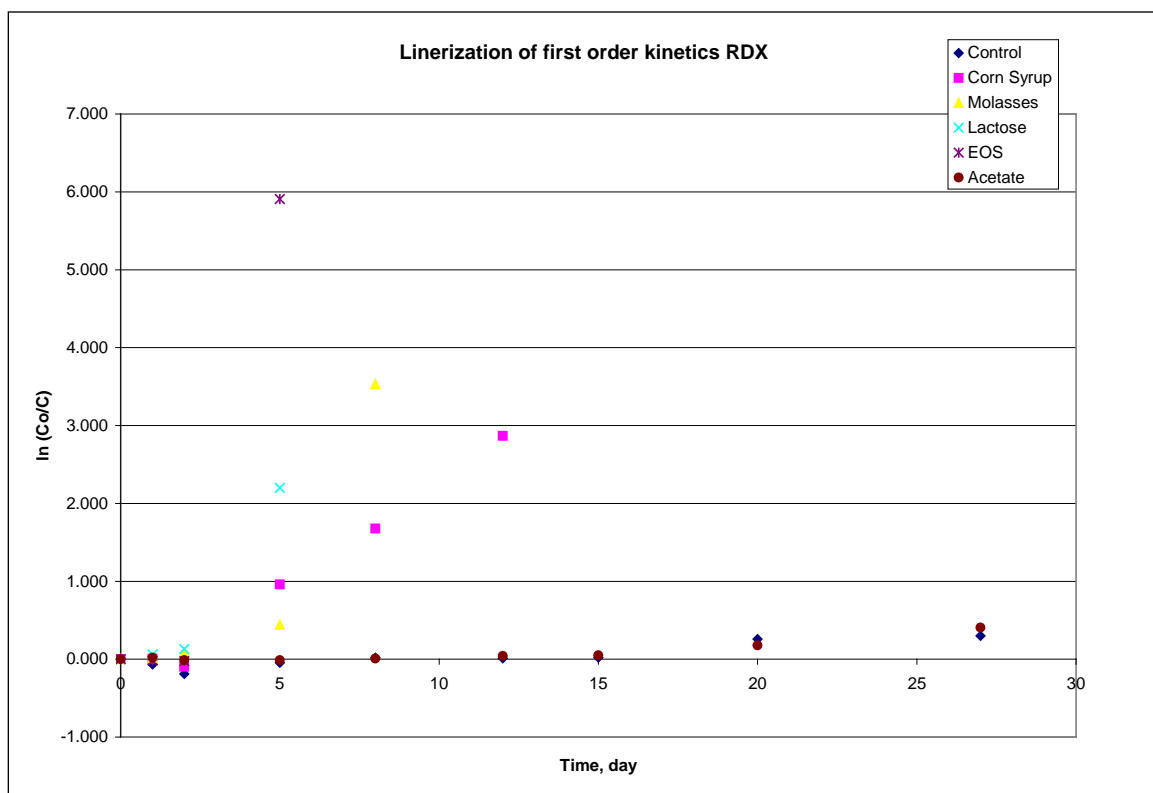


Figure B2. Plot of first-order function linearization RDX Run 1.

Table B3. TNT Run 2

TNT	TNT Control	TNT Benzoic Acid	TNT Ethanol	TNT Whey	TNT Na Lactate	TNT L. Soil Lactose	TNT Dead Control	TNT L. Soil Control
0	1.005	1.020	1.083	0.996	1.023	1.063	1.143	1.120
2	0.651	0.825	0.623	0.002	0.725	0.002	0.918	0.905
7	0.194	0.337	0.180	0.000	0.190	0.000	0.541	0.766
15	0.000	0.000	0.000	0.000	0.000	0.000	0.415	0.723
24	0.000	0.000	0.000	0.000	0.000	0.000	0.136	0.306
In (CO/C) Time	Control	Benzoate	Ethanol	Whey	Na Lactate	L. Soil Lactate	Autoclaved	L. Soil Control
0	0	0	0	0	0	0	0	0
2	0.43339	0.211771	0.552717	6.210265	0.344189	6.276017	0.219869	0.213149
7	1.644553	1.108465	1.796695		1.685552		0.748284	0.379467
15							1.013425	0.437214
24							2.1266	1.29641
k	0.2336	0.1544	0.2582	3.1051	0.2356	3.138	0.0842	0.0477
r ²	0.9992	0.9853	0.9992	1	0.989	1	0.9636	0.8811
t.5	2.97	4.49	2.68	0.22	2.94	0.22	8.23	14.53

Table B4. RDX Run 2.

RDX	RDX Control	RDX Benzoic Acid	RDX Ethanol	RDX Whey	RDX Na Lactate	RDX L. Soil Lactose	RDX Dead Control	RDX L. Soil Control
0	0.815	0.806	0.866	0.764	0.788	0.737	0.653	0.623
2	0.813	0.803	0.782	0.473	0.787	0.073	0.553	0.535
7	0.751	0.754	0.729	0.000	0.751	0.000	0.693	0.580
15	0.772	0.809	0.756	0.000	0.728	0.000	0.545	0.583
24	0.631	0.697	0.387	0.000	0.303	0.000	0.487	0.509
In (CO/C) Time	Control	Benzoate	Ethanol	Whey	Na Lactate	L. Soil Lactate	Autoclaved	L. Soil Control
0	0	0	0	0	0	0	0	0
2	0.002457	0.003315	0.101219	0.479741	0.000847	2.31675	0.166127	0.152903
7	0.081339	0.065836	0.172284		0.048113		-0.059423	0.071518
15	0.053772	-0.004541	0.1359		0.078316		0.18069	0.065788
24	0.256411	0.145363	0.805937		0.956443		0.293139	0.202099
k	0.0088	0.0046	0.0267	0.2399	0.0287	1.1584	0.0113	0.0078
r ²	0.8014	0.5326	0.7581	1	0.6772	1	0.5092	0.1274
t.5	78.77	150.68	25.96	2.89	24.15	0.60	61.34	88.87

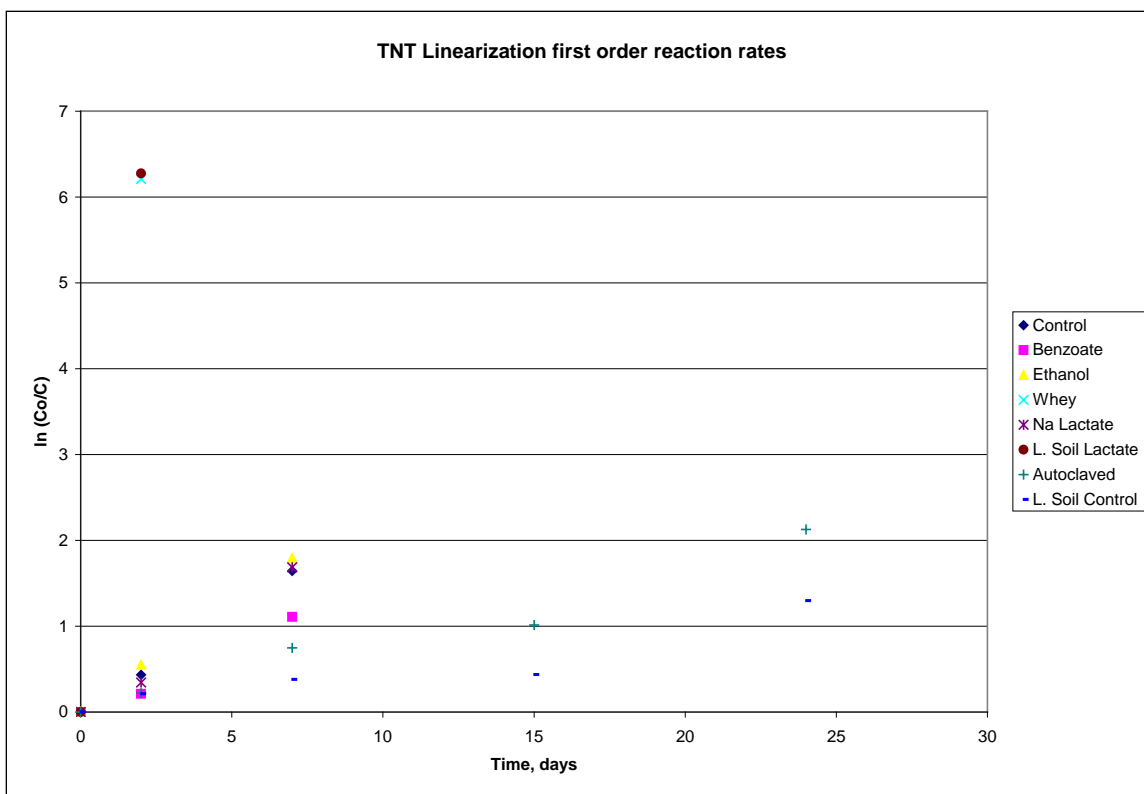


Figure B3. Plot of first-order function linearization TNT Run 2.

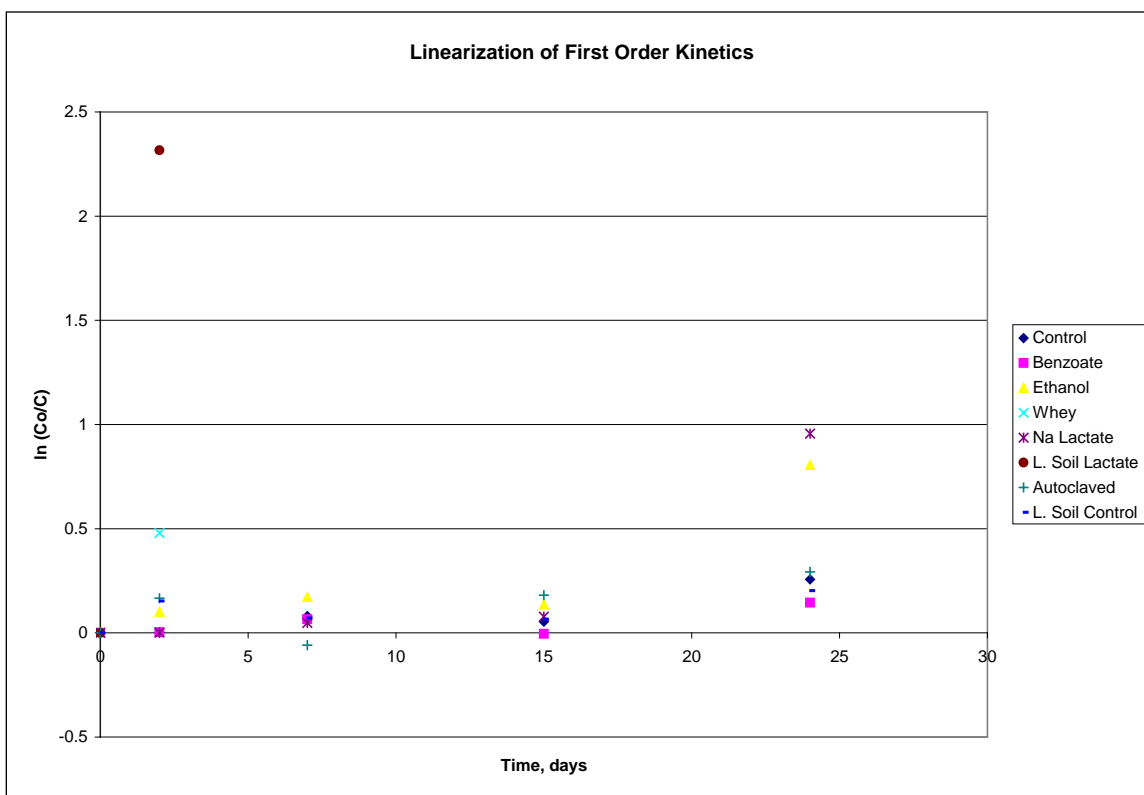


Figure B4. Plot of first-order function linearization RDX Run 2.

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1. REPORT DATE (DD-MM-YYYY) January 2012		2. REPORT TYPE Final report		3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE The Results of a Laboratory Feasibility Study for the Biological Treatment of Umatilla Groundwater				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Victor F. Medina. Heather Knotek-Smith, David Gent, and Agnes Morrow				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Army Engineer Research and Development Center Environmental Laboratory 3909 Halls Ferry Road Vicksburg, MS 39180-6199				8. PERFORMING ORGANIZATION REPORT NUMBER ERDC/EL TR-12-2	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Headquarters, U.S. Army Corps of Engineers Washington, DC 20314-1000				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The Umatilla Chemical Depot (UMCD) has been slated to close as an Army facility under the Base Realignment and Closure (BRAC) Program. One remaining environmental issue is a groundwater plume contaminated with explosives; the two most critical are 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX). Since 1994, a groundwater pump and treat system has operated at the site as a corrective measure for the contaminated groundwater. The effectiveness of this treatment system has plateaued, and it appears that the system will not meet the long-term treatment goals for the site. This study was conducted to evaluate the potential of bioremediation as a means of optimizing the performance of the groundwater treatment system. Groundwater from the site was collected through the groundwater pump and treat. Soil was collected from the wash out lagoon area, which is the primary source area for most of the contamination. These were used to set up microcosm studies to evaluate the biodegradation of the contaminants. Microcosms were set up using 1-liter Erlenmeyer flasks. The groundwater was spiked to about 1.2 and 0.8 mg/L of TNT and RDX, respectively. The flask had 200 g of Umatilla soil (some experiments had 50 g) and 500 mL of spiked groundwater. Various treatments were assessed; i.e., various organic amendments were used as co-substrates to stimulate the degradation of TNT and RDX. Nine amendments, as well as various unamended samples, were tested. The reactors were incubated over a 27- day period under an anaerobic hood. Removal of the contaminants was measured, as was the formation and removal of transformation products, changes in pH, Total Organic Carbon, Eh, and dissolved oxygen. TNT was relatively easy to degrade, as it removed even many of the controls. Presumably the anaerobic conditions under the hood were enough to stimulate degradation. RDX, on the other hand, was more difficult to treat. The best amendments were molasses, corn syrup, emulsified oil (EOS), lactose, and whey.					
15. SUBJECT TERMS		Biological treatment of groundwater		Groundwater treatment system	
Amendments		Bioremediation		RDX	
Base Realignment and Closure Program		Co-substrates		TNT	
		Degradation		Umatilla Chemical Depot	
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED		69	